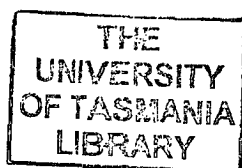


Aspects of *Vitis Vinifera* L. cv Pinot Noir
Fruitfulness in Tasmania

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Submitted in fulfilment of the requirements for the degree of
Doctor of Philosophy

School of Agricultural Science
University of Tasmania

Declarations

This thesis contains no material that has been accepted for a degree or diploma by the University of Tasmania or any other institution. To the best of my knowledge and belief this thesis contains no material previously published or written by another person except where due acknowledgement is made in the text of the thesis.

A handwritten signature in black ink, reading 'J E Heazlewood' in a cursive style.

Joanna E. Heazlewood

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Joanna E. Heazlewood

Acknowledgments

During the course of this project I was supported by a Tasmanian Institute of Agricultural Research scholarship and a Grape and Wine Research and Development Corporation scholarship. Substantial in-kind support was provided by the University of Tasmania's School of Agricultural Science as well as Home Hill Wines, Meadowbank Wines, Bream Creek Vineyard, Pipers Brook Vineyard, and BRL Hardy in the form of experimental sites, plant material and technical assistance.

Many people have helped make this project a success:

- The Vineyards Association of Tasmania and the Tasmanian Pinot Noir Growers, for their enthusiasm for and endorsement of this project;
- Dr Steve Wilson, Dr Alistair Gracie and Professor Rob Clark from whom I have learnt so much;
- Drs Phil Brown and Linda Falzari, and Professor Robert Menary, for advice and support whenever requested;
- Dr Noel Davies for generous and expert assistance with HPLC and MS analysis;
- Dr David Steele for his expert knowledge and assistance in Scanning Electron Microscopy;
- Dr Cameron Spur and Ann Gaffney for laboratory advice and assistance including FDA solution, analine blue solution and pollen germination techniques;

- Dr Mark Krstic for assistance with carbohydrate extraction techniques;
- Messrs. Phil Andrews and David Wilson for technical support at the agricultural science horticultural research station;
- Dr Ashley Townsend for leaf nutrition analysis;
- Dr Peter Gore for pollen exclusion techniques;
- Mr Bill Peterson and Mrs Angela Richardson for technical support at the School of Agricultural Science.

Primarily I have to thank my family: Robert, Lyn, and Anthony Heazlewood for being my family, particularly Robert who assisted with time management and acted as a great sounding board.

Also my thanks goes to my patient partner, David Jones.

Abstract

The issue of yield variability and the physiological factors that determine its severity and patterns of year to year fluctuation remain much of a mystery in the cool climate wine-producing region of Tasmania. Research into causes and management has generally focused on the influence of one specific factor, or issues within any one growing season, with little attention to the full cropping cycle.

Yields of both Pinot Noir and Chardonnay over an eleven-year period were analysed for correlations between vineyards and for consistent responses to seasonal weather. Fluctuations in yield across the industry showed no consistent patterns, possibly because available data corresponded with a period of rapid growth and change throughout the region. However, yields from two vineyards with established and uniform vine age and management, were correlated with each other and with weather events corresponding with previously identified periods of sensitivity in the reproductive growth cycle. At one site a multiple regression model of yield against mean maximum daily temperature and mean sunshine hours at flowering accounted for 90% of the variation in yield. At the second site, sunshine hours and rainfall at flowering accounted for 76%, with sunshine hours alone accounting for 63%.

Due to the effects of weather at flowering on yield and uncertainty surrounding the method of pollen transfer and cross and self-pollination in *Vitis vinifera*, a detailed study into the mode of pollination in Pinot Noir was conducted. The stigmas of both open and closed flowers were examined using scanning electron microscopy and

fluorescence microscopy for evidence of anthesis and pollen tube growth, and pollen viability tests were carried out on pollen from both open and closed flowers. Pollen was shown to be present and viable on stigmas prior to cap fall, however pollen tubes did not begin to grow until after cap fall. Bagging of inflorescences throughout flowering in field vines, found that fruitset was not related to cross-pollination.

In view of the effect of cap fall on pollen tube growth, the common commercial practice of fungicide application during flowering was investigated. Dilutions of commercial formulations of three fungicides were added to liquid germination media and pollen germination rates were measured. One of the fungicides was also used in a field study where vines were sprayed during flowering at 5% and 80% cap fall. All three fungicides prevented pollen germination at the recommended concentration for field sprays in the *in vitro* experiment, with pollen tube growth resuming at greater dilutions. In the field, percent fruitset was not affected by fungicide application at either stage of flowering, however average seed number at harvest was affected by fungicide application with the 5% cap fall treatment having a significantly lower seed number.

As recent research suggests that bunch number is the yield component that contributes most to yield variation in cool climate wine producing areas, one option to manage yields from vines subject to variable spring weather, is to dormant prune to a yield target above the desired level, then summer prune or bunch thin to achieve the desired yield after fruit set. Different bud numbers were imposed on field grown vines over three seasons, varying from canopies of ten to forty buds. Yield

components were measured at each harvest. Vines with smaller bud numbers had greater over-wintering starch levels, fruit had a lower pH and anthocyanin content, but there was no effect on sugar content. In a second field trial with the same pruning treatments, bunches were removed (thinned) at veraison, to create fruit loads equivalent to industry levels for small, medium and high yields. There was a reduction in berry number per bunch at the heaviest fruit load, but no treatment effects on bunch weight or berry weight.

In both field trials bunch number per bud decreased with increasing number of buds retained in winter pruning, and berry number per bunch also decreased. Yield in the previous season also influenced bunches per bud in the current season. Results are discussed in terms of the influence on yield, of carbohydrate reserves and current photosynthesis in the period from bud burst to flower initiation, and on the potential to manage yield using bunch thinning or shoot thinning of vines dormant pruned to bud numbers locally regarded as overcropping. In particular the view that bunch number is the primary yield component is questioned. The results lead to a conclusion that bunch size is the main factor influencing yield variability, and contrary to most published literature bunch size, rather than yield, may be an important influence on wine quality.

Publications resulting from thesis

Chapter 3:

Heazlewood, J.E., and Wilson, S., 2004, Anthesis, pollination and fruitset in Pinot Noir, *Vitis*, 43, (2), 65-68.

Chapter 3:

Heazlewood, J.E., Wilson, S., Clark, R.J., and Gracie, A., 2005, Pollination of *Vitis vinifera* L. cv. Pinot Noir as influenced by Botrytis fungicides, *Vitis*, 44, (3), 111-115.

Chapter 2:

Heazlewood, J.E., Wilson, S., Clark, R.J., and Gracie, A., 2004, Key climatic effects on the yield of Pinot Noir in Tasmania. Poster presentation at the 12th Australian Wine Industry Technical Conference, Melbourne, July 2004.

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Chapter 1 - Introduction

The Australian wine industry has been expanding rapidly since the late 1980's and is currently one of the most important horticultural industries in the country. In 2003 the industry crushed 1.41 million tonnes of grapes to make 919 million litres of wine. The total production area included 151 000 hectares of bearing vines, with a further 15 000 hectares of non-bearing vines (Anon, 2003). Production is centred in regions of New South Wales, Victoria, Western Australia and South Australia where favourable cool winter and warm summer climatic conditions exist, but the production area extends from highland areas in subtropical Queensland to cool temperate regions in the southern most state of Tasmania.

Although only a small component of the Australian industry, production in Tasmania has also increased dramatically in the past 5 years from 3113 tonnes in 1998 to 5198 tonnes in 2003 (Farquhar, 2004) and is continuing to expand. The most important varieties are Riesling, Chardonnay, Sauvignon Blanc and Pinot Noir, with 878 tonnes of the latter produced in 2002, which accounted for 35% of the total (Farquhar, 2004). With most of the Australian wine regions too warm for production of quality Pinot Noir, the dominance of this variety is apparent only in Tasmania and a few other localised cool climate locations on mainland Australia.

Tasmania is located at latitude 41° - 43° South, corresponding with the northern latitude of the classic cool-climate areas of Burgundy and Bordeaux in France. The local industry has utilised the suitability of the cool temperate climate for Pinot Noir

to promote particular wine styles, described, for example, as the “elegant and intensely flavoured wines of today, with rich colour and characteristic varietal aromas” (Johnstone, 2000).

While Pinot Noir wine quality in cool temperate regions can be high, the yield potential of vineyards is generally lower compared with other varieties in warmer regions. Warmer regions of Australia produce commercially acceptable wines well below the cost of Tasmanian production. Local vignerons have been primarily interested in producing premium quality wines for high value niche markets, with less emphasis on yield in comparison to other regions. Recent structural changes towards larger scale vineyards with boutique vineyards accounting for a smaller proportion of total production have resulted in a more commercial approach.

Vignerons have become increasingly concerned about low yields with a high year to year variability and emphasis is therefore shifting to a balance between yield and quality.

The evaluation of wine quality remains subjective and opinions on quality vary from taster to taster. However as noted by Jackson and Lombard (1993) there are common objective criteria for assessing fruit quality including; sugar concentration, titratable acidity, pH, colour intensity, flavour or aroma potential, as well as the absence of negative specifications. Techniques such as the glycosyl glucose assay which measures the amount of flavour present in grape juices, the fourier transform infrared spectroscopy technique which provides correlative measurement of compounds that are present in relatively low concentration in grapes and high performance liquid

chromatography combined with spectrometric methods detect numerous potentially unknown red pigments in wine. These systems are employed in laboratories and wine making facilities and are often used to determine the suitability of grapes to make wine of a particular price bracket or wine style. However many of these techniques have been developed for warmer climate varieties and their use for measurement of quality in cool climate Pinot Noir was questioned by Farquhar (2003). Few reports on quality determination for Pinot Noir use measures other than total soluble solids, pH, titratable acidity, and berry colour.

The distribution of viticultural areas around the world and quality of wines produced in the regions confirms a relationship between climate and quality. Jackson and Lombard (1993) suggests as a general rule that providing there is sufficient heat accumulation to ripen a specific grape cultivar, quality will be inversely related to average temperatures and length of the growing season. For this reason quality table wines from Pinot Noir are produced only in cooler central and northern areas of France and coastal parts of Australia and New Zealand (Halliday, 1998) and recent increases in production from Chile (Anon, 2005) also confirm the limited climatic range for this variety.

As already noted, wine quality depends greatly on maturation temperature (Gladstones, 2000), and the best variety in a region will be one which suits the length of the growing season. Maturation should occur under relatively cool conditions but at temperatures sufficient to continue sugar accumulation and flavour development in the berries. The maturation characteristics of Pinot Noir appear well matched to the

Tasmanian climate, with the area gaining an international reputation as a producer of high quality Pinot Noir. While quality standards are high and wines are improving in both quality and consistency, profitability remains low and variable. Yields are variable across the industry with some vineyards reporting an annual variation as high as 70%. Even with low target yields intended to maximise quality, year to year variability is extreme and there are no published reports indicating an inverse relationship between yield and quality. A recent report (Farquhar, 2003) did suggest such a relationship, but the results were inconclusive and further work is needed to ensure that growers do not sacrifice profit, rather than quality, by setting inappropriate target yields.

Throughout most wine regions in the world year to year fluctuations in crop yield can have significant impacts on grower returns and adverse effects on intake scheduling, storage requirements and marketing strategies for domestic or export markets. While growers and wine makers are aware of the problem, solutions have generally targeted yield prediction to improve vintage management (Clingeleffer *et al.*, 1997) rather than vineyard management to stabilise yield. There are few research publications reporting management options to reduce yield variability in spite of the fact that early publications (Winkler *et al.*, 1974) have identified environmental influences on vine growth likely to impact on critical stages in crop development. Unfavourable weather at critical times in the growing season is often blamed by growers for low yields, but locally there is a lack of evidence showing links with particular weather patterns. In addition growers generally do not adjust management practices in response to “unfavourable” weather. Vineyard factors including biennial

bearing (Watson, 2000) and pest and disease influences on yield are also poorly understood.

Certain stages in the flowering and fruiting cycle are more sensitive to detrimental environmental effects than others (Dunn, 2003). The likely magnitude of an environmental effect on final yield will depend on conditions at particular stages in development. Although information on phenology and vine physiology at various stages of development have been published (for example Coombe and Dry, 2000) there seems to be little recognition by growers of the potential influence of conditions in one season on yield in both the current and subsequent seasons. This difficulty may be due to a lack of information specific to the local industry.

Yield records and growers comments confirm that vines managed to a conservative target yield, produce well below target yields in all but exceptional seasons. Vines pruned to a target based on bunch number subject to negative influences on bunch emergence, bunch size and berry weight almost inevitably produce below target yields.

This strategy appears to have given rise to a widespread concern about excess vine vigour. Earlier local work by the present author (Heazlewood, 2000) confirmed that, in Tasmanian Pinot Noir vegetative growth and yield were inversely related, suggesting that conservative yield targets may be contributing to the excess vigour problem rather than the reverse.

In the variable climate of Tasmania, vineyard management practices have been built around European influences. It is difficult to justify the use of a management model in Tasmania based on more predictable weather patterns of the continental climate of western Europe. The climate of the Tasmanian wine region was described by Langford in Davies (1965) as a temperate marine climate, broadly influenced by heat absorption and storage by the surrounding sea, producing abnormally mild winters and cool summers for the latitude (Langford, 1965). Gladstones (1992) described all Tasmanian viticultural climates as cool and highly maritime, with only small temperature differences between winter and summer compared with classic cool viticultural climates of northwestern and central Europe. In Tasmania, the critical times of flower initiation, appearance and anthesis in spring / early summer, and the last stages of fruit maturation in autumn do not occur in a period of sharp seasonal temperature change. These periods are marked by highly variable weather and a gradual shift in temperature between relatively mild summer conditions and a similarly mild winter. Long term mean monthly temperature and rainfall data for Hobart in Tasmania's south and Launceston in the north are presented in Table 1.1.

Table 1.1 Long term average mean monthly maximum and minimum temperatures (°C) and rainfall (mm) for Hobart and Launceston airports. The long term average is based on 48 years of data for Hobart and 66 years of data for Launceston.

		Max. Temperature	Min. Temperature	Rainfall
January	Hobart	11.4	21.5	40.2
	Launceston	9.3	22.7	43.2
February	Hobart	11.6	21.8	36.3
	Launceston	9.7	22.9	39.2
March	Hobart	9.9	19.6	36.4
	Launceston	7.7	20.8	37.5
April	Hobart	9.9	20.0	44.4
	Launceston	7.4	18.6	54.3
May	Hobart	7	14.2	35.9
	Launceston	3.2	13.3	60.9
June	Hobart	5.2	12.9	68.2
	Launceston	3.5	11.6	63.1
July	Hobart	4	12.2	44.8
	Launceston	2	10.7	78
August	Hobart	4.1	13.1	48.1
	Launceston	2.4	12.1	77.1
September	Hobart	6.4	15.5	39.9
	Launceston	4.3	14.7	63.5
October	Hobart	7.1	18.0	47.6
	Launceston	4.9	17.1	61.8
November	Hobart	10.5	19.5	44.4
	Launceston	7.3	18.5	50
December	Hobart	11.5	21.1	53.6
	Launceston	9	22.8	50.4

Gladstones (1992) suggested that as a result of the maritime temperature pattern, vine phenology in Tasmania can be expected to be influenced more strongly by both weather patterns and altitude than in almost any other wine region. Gladstones (1992) used a mean monthly temperature of 19°C as the cut-off mean temperature above which, on average, there is no further increase in the rate of physiological development, through the growth and ripening period. Australian Bureau of Meteorology records show that long term average mean monthly temperatures in Tasmania are below this temperature throughout the year. Consequently temperature

effects on vine phenological development accumulate over the whole season and differences between sites even within the one locality may be dramatic. The temperature difference resulting from a simple altitude difference of 40m would have a dramatic effect on time of ripeness due to the fact that a difference of 0.24°C (based on the estimated lapse rate) in mean temperature is equivalent to 50 effective day degrees up to ripeness (Gladstones, 1992).

As noted, the issue of yield variability in the Tasmanian wine industry is one of increasing concern as individual vineyards become more commercial, shifting away from lifestyle or hobby based enterprises. Any advance towards more stable yields and more accurate achievement of targets will benefit growers, contract wine makers and planning for improved market development.

This project aimed to identify major sources of variation in yield of Pinot Noir and determine the critical vine growth cycle stages which should be targeted for development of management strategies to reduce yield variability. As there is little published work on management of yield variability the study was developed from recent work on yield prediction by an Australian study group (Clingeleffer *et al.*, 1997; Martin *et al.*, 2000). These studies concluded that the seasonally variable determinants of yield were, in decreasing order of contribution to variability, bunch number (60%), berry number (30%), and berry weight.

Initially Tasmanian industry yield records were used to study local yield variability in detail, concentrating on inter-site variability and possible influences of seasonal

conditions at critical times during development. Critical periods of vine development were taken as follows: floral initiation and initial floral differentiation, bud burst, fruit set and grape maturation with timing based on Coombe and Dry (2000).

From these results and work by Gladstones (2000) and the yield prediction studies of Martin *et al* (2000) subsequent chapters examined both environmental and selected management effects on the primary yield determinant, number of bunches and the expected secondary influences of fruitset and berry size. Management factors influencing the number of bunches included pruning, crop regulation and canopy structure and the effect of disease control chemicals at flowering on the number of berries. The effect of pruning on stored carbohydrate in the canes over winter was also studied, and the relationship between fruit yield and carbohydrate levels examined.

Overall this study was not primarily concerned with wine quality, however economic viability of local vineyards will remain dependant on maintaining a balance between yield and market value of wine. Consequently, in one of the trials dealing with crop load, relationships between fruit yield and several basic quality parameters were studied.

Results are discussed in relation to uncertainties about several aspects of vine growth and development for this variety in cool climate conditions and practical implications for improved management towards target yields.

Chapter 2 - Yield Variability and Climate - Historical Data

Introduction

The issue of variable grape yields in Tasmania affects most grape growers, and industry has identified the problem as priority for research. While industry concerns about variable yields with Pinot Noir are based mainly on comments made by leading growers, there is some documented evidence verifying major year by year variations at grower, regional and state levels (Farquhar, 2004). Until 2003/4, Tasmanian vineyards had a statutory requirement to annually report yields to the State Liquor Licensing Board, which has a record of yields and planted area by variety for all vineyards in Tasmania since 1990. Whilst limited, in that they give no information on the yield components and do not take clonal, management, or planting age issues into account, these accurate public records present the annual yield on individual properties as well as averages within regions.

Analysing this raw data to identify any yield patterns that may be evident on a state, regional or individual property level may provide some useful information about the nature of yield variability in Tasmania. Correlating such yield data with seasonal climate or significant weather events at periods of known sensitivity in the grapevine growth cycle, may also provide some insight into the factors influencing yield variability. There are however five main limitations in using the data set i) the timing of the critical stages of the reproductive cycle identified by local viticulturists or extrapolated from literature based on studies in other regions may be inaccurate, ii)

variable topography and distance of the vineyards from the weather station may be too great to accurately describe the local microclimate at any particular site, iii) rather than the average weather having an effect on yield it may be specific one-off local weather events such as frosts which have had an impact, iv) changes in training, canopy management, disease control, short term or other management issues such as bunch thinning, may contribute to a lack of correlation with weather and v) inclusion of all producing vineyards, regardless of age, may unduly influence both property and regional figures in a rapidly changing industry.

However, with these limitations in mind the analysis of yield variability was still considered worthwhile in an attempt to identify any trends that may exist, and to confirm that the problem of yield variability is a real issue in Tasmania.

The components of yield

Fruit yield is made up of different components which when combined result in a yield usually reported as tonnes per hectare. The components of yield include the number of vines per hectare, number of canes per vine, number of buds per cane, number of bunches per bud, bunch weight, number of berries per bunch, and individual berry weight. The number of vines per hectare is determined at planting, while the number of canes per vine, under the cane pruning systems generally used in Tasmania, is determined at pruning.

Aside from the number of vines per hectare and the number of canes per vine, all yield components may be influenced by management practices as well as climate, during vine growth flowering and fruit development.

The grapevine growth cycle

The cycle of vegetative and reproductive growth in grapevines has been thoroughly investigated and the timing of various stages well reported for major wine producing regions. The accepted growth cycle for warmer Australian regions is given in the following diagram (Figure 2.1), taken from Coombe and Dry (2000).

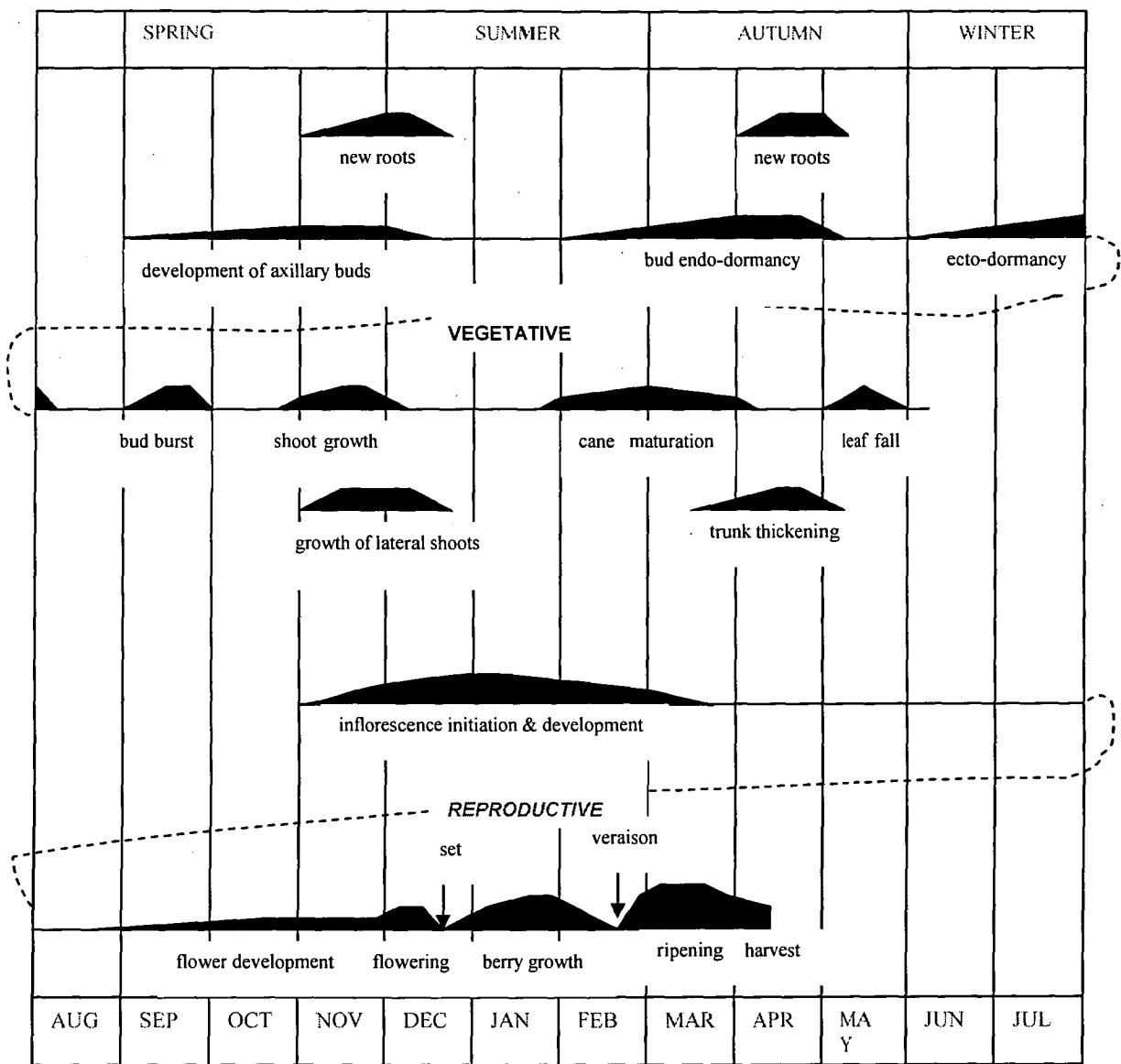


Fig 2.1 Cycle of vegetative (top) and reproductive (below) growth events in the grapevine in relation to months of the year in the Southern Hemisphere. (Coombe and Dry 2000)

The growth cycle has also been described as follows by Mullins *et al* (1992): The first formed bud that arises in each leaf axil of current season shoots is known as the prompt bud. The prompt bud grows out in the season of its formation to produce a short lateral shoot off the main shoot. This “summer lateral” is less fruitful than

primary shoots, with bunches carried on these summer lateral shoots known as the second crop. Clusters on these shoots present problems as they flower, set fruit and mature later than the rest of the crop. The first leaf on this summer lateral is reduced to a prophyll and the bud formed in the axil of this prophyll is the latent bud. The latent bud grows slowly during spring, developing 6 to 10 leaf primordia and up to three inflorescence primordia before entering dormancy (Mullins *et al*, 1992). This latent bud is a compound bud consisting of buds in each of the leaf axils. The primary latent bud is first formed and accessory buds usually remain small and seldom contain inflorescence primordia.

Lateral shoots elongate from the overwintering latent bud formed during the previous growing season (Gerrath, 1993). However each branch is not entirely preformed, as sections of the distal portion grow out in the season they are formed. These factors mean that the primary latent buds of the distal portion of the shoot normally grow in the year they are initiated and may or may not possess inflorescences. Accessory buds normally remain dormant and become overwintering buds on the distal portion and form next years crop. However in some cases these high order buds are fruitless. The first order buds in the basal region of the branch do not grow out in the year they were formed, as they were part of an overwintering latent bud.

The formation of inflorescences and flowers involves three well-defined stages; formation of Anlagen, formation of inflorescence primordia and finally differentiation and expansion of flowers (Srinivasan and Mullins, 1981). Anlagen are protuberances, arising from the apices of latent buds. These uncommitted primordia,

can form an inflorescence, a tendril or a shoot and always occur opposite a foliage leaf (Mullins *et al.*, 1992). Anlagen, which become an inflorescence, undergo repeated branching to form a conical structure composed of many branch primordia. These floral initiation processes occur in the year preceding the appearance of flowers. The timing and subsequent rate of development of the flower cluster depend on the position of the bud on the cane and the position of the inflorescence on the condensed shoot within the bud (Pratt, 1971).

The final stages of floral differentiation occur just before and during budburst in the spring of flower emergence. During this time the flowers are formed and continue to develop through to anthesis. Based on the timing of events it is widely accepted that inflorescence initiation occurs before bud dormancy and that floral differentiation takes place post-dormancy. Gerrath (1993) reported that some authors supported the view that floral differentiation actually takes place before dormancy in autumn, but all research since the early 1970's support the former theory.

The flowers emerge in early spring. The young inflorescence is compact, and continues to grow until the time of bloom, when it reaches maximum size (Lavee, 1985). The number of flowers per inflorescence varies with variety as does the compactness of the inflorescence.

In many perennial species where flower initiation occurs during or before the development of a current crop there is an interaction between the two, resulting in alternating heavy and light crops in successive seasons, often known as biennial

bearing or alternate bearing. This phenomenon occurs in many temperate fruit tree crops including apples and several *Prunus* species (Westwood, 1978). Poor floral initiation is a feature of the low yielding year in species or cultivars showing biennial bearing. Both low carbohydrate levels in the branches, and high gibberellin levels have been considered the basis of biennial bearing cycles (Watson, 2000). Biennial bearing has been recognized and managed in fruit trees but has rarely been discussed as a factor influencing grape vine yields. Recently, data on yield fluctuations consistent with biennial bearing patterns have emerged and are gaining some recognition, with Watson (2000) reporting biennial bearing in *Vitis labrusca*.

Most grapevine cultivars are highly floriferous and inflorescences are formed at most nodes along the cane. Each inflorescence may contain up to a thousand flowers. Of these flowers, 70-80% normally fail to develop into mature fruits and abscise. The term 'fruitset' is used to describe the successful transformation from flower into developing fruit (Mullins, 1992).

In most cases fertilisation occurs 2 or 3 days after pollination (Pratt, 1971). There are three ways by which flowering can lead to ovaries of commercial cultivars becoming berries; simulative parthenocarpy, stenospermy or 'normal development' (May, 2004). In simulative parthenocarpy, pollination without egg nucleus fertilisation leads to seedless small berries. In stenospermy, pollination and fertilization is followed by seed abortion leading to seedless berries. However, for most flowers that develop into fruit, pollination and fertilisation lead to continuing seed development and berries are therefore considered normal (May, 2004). Pinot Noir flowers have

four internal ovaries, and therefore individual berries can have a maximum seed number of four. The number of seeds per berry has been shown to affect berry size, and is generally thought to be linked to giberrellins produced by the seeds (Branas and Vergnes, 1963); cited in May, 2004). Once a flower is fertilised the formed ovary is ready to undergo a series of transitional phases to become a mature berry. The growth of a grape berry is comprised of two distinct phases. The primary phase, often termed berry formation, begins with a time of cell division which largely determines the berry's final size and shape. This phase of cell division transforms gradually into a period of cell enlargement that tapers off as the first sigmoid cycle ends. At the completion of this phase the berry is green and hard (Coombe and McCarthy, 2000). The onset of sugar accumulation marks the start of the second cycle, termed veraison, in which the berry also begins to soften, colour and increase in size. During this second cycle hexose sugars accumulate in the skin and flesh, phenolics and potassium build up in the skin, and flavour compounds accumulate late in ripening (Coombe, 2000).

Management and weather effects on yield components

Bunch Number

As noted earlier, the number of bunches per vine is a primary determinant of yield. Martin *et al* (2000) found, after analysing grape yield data throughout Victoria (Australia), that the largest source of seasonal yield variation was bunches per vine, accounting for approximately 60% of the variation. The number of bunches which form per bud is genetically determined. Although there may be some compensatory effects, varieties such as Cabernet Sauvignon are generally regarded as low yielding

and varieties such as Muller Thurgau high yielding (Jackson and Steans, 1983-4). However, accepting this predetermined limit, there are factors that will have an impact on bunch number and therefore have a large effect on final yield. The number of bunches per bud has been shown to be influenced by the light intensity and temperature as the bud is being laid down in the previous season, as well as carbohydrate availability in both the previous and current seasons (Buttrose, 1969a). Warmer temperatures encourage higher bunch numbers per bud, as does higher light intensity. Low carbohydrate status during inflorescence initiation can also reduce the bunch number in the following spring (Priestley, 1962), and there have been some suggestions of an inverse relationship between the level of bud burst and current season carbohydrate availability (Mullins *et al*, 1992). Given that both a high rate of bud burst, and bunch number per viable bud are crucial for achieving maximal bunch numbers the level of current season carbohydrate availability may also influence bunch numbers.

Within genetic limits, floral initiation determines the number of fruitful buds, and potential number of bunches per bud. The number of potentially fruitful buds per vine is generally managed by pruning during dormancy (Coombe and Dry, 2000). The proportion of potentially fruitful buds that actually break dormancy and bear fruit depends heavily on the variety and its interaction with weather (Barnard, 1932). In some varieties a large proportion of the buds fail to burst, but the number can also vary considerably from season to season. In addition, the chances of the successful development of a bud are governed by position on the cane. The proportion of terminal buds that give rise to shoots is high, whilst a comparatively large number of

basal buds remain dormant (Barnard, 1932). Consequently, routine pruning to a given bud number will invariably result in variable bunch number (Dunn *et al*, 2001). A seasonally variable proportion of selected buds remaining dormant, producing only a vegetative shoot, or fewer than normal bunches per bud will generally result in bud numbers lower than the target or genetic potential.

Bud development determines the potential number of shoots per node, the number of inflorescence primordia and thus potential bunches per shoot (Dry, 2000). Dry (2000) suggested that the term node fertility is a more meaningful measure than bud fruitfulness as any effect of canopy management will not be seen in bud fruitfulness, but rather the response is in terms of an effect on budburst, i.e. , shoot number per node.

A number of treatments have been shown to alter bud fertility. For example, Menary (1979) showed that applications of CCC [(2-Chloroethyl)-trimethyl ammonium chloride] at bud burst in the current season led to an increase in the number of bunches per vine and number of berries per bunch. Bennett *et al* (2002) showed that the number of inflorescences per shoot and hence the resulting number of bunches per shoot were reduced by defoliation at regular intervals from 4 weeks after bloom in the previous season. The authors concluded that the reduction in bunch numbers was due to lower available reserve carbohydrates at bud burst.

Berry Number

Berry number per bunch is determined by (i) the number of flowers present at anthesis, (ii) the proportion of these that set fruit successfully and, (iii) the percentage

of berries that remain attached until maturity (Dunn and Martin, 2000). Therefore an upper limit for berry number per bunch is set during initiation in the previous season. At this time, the number of flowers initiated per bunch can be influenced by temperature, light intensity, water availability, carbohydrate status and plant hormones (Srinivasan and Mullins, 1981). Priestley (1962) reported that there was no direct evidence to suggest that, for fruit trees, the carbohydrate requirements of blossom buds during their initiation are greater than that of vegetative buds. It was concluded that, while plants require the presence of adequate carbohydrate supplies for bud development, flower initiation was generally induced by some other mechanism acting in response to an environmental cue (Priestley, 1962).

The number of inflorescences per shoot has also been shown to effect the number of flowers per inflorescence and hence the number of berries per bunch in Cabernet Sauvignon (Dunn and Martin, 2000). Environmental conditions during inflorescence initiation in grapevines control flower numbers per shoot and per vine in two ways. First through effects on inflorescence numbers per shoot and secondly, a finer control may exist where conditions favouring an increase in the number of inflorescences per shoot, may also promote higher flower numbers per inflorescence (Dunn and Martin, 2000).

The final berry number per bunch is influenced by pollen transfer, fertilisation and berry development, with the proportion of flowers setting fruit referred to as “fruitset”. A high percent fruitset has been correlated with successful pollination and seed development (May, 1992). Vine nutrition can play a role in flower development

and fruitset. Skinner and Matthews (1989) demonstrated that initiation, differentiation and maintenance of reproductive primordia were all sensitive to phosphorous supply throughout the season and Singh and Rethy (1996) revealed that bunch weights increased with boron foliar sprays, due to increased berry numbers. Zinc deficiency seems to retard berry development in grapes, and manifests in symptoms like straggly fruit clusters with poor fruitset and variable berry size (Sharma *et al.*, 1995). Generally these studies on mineral nutrition do not clearly indicate whether the effects are direct on berry development or indirectly on pollination and fertilisation.

Berry Size

Whilst fertilisation is critical for fruitset, auxin is produced in developing seeds and is thought to promote cell division and expansion in the developing berry (May, 2004). Therefore the greater the number of seeds, the greater the cell expansion of the berry and hence the larger the resulting size. The level of starch stored in the trunk and roots and canes going into winter appears to be important for berry development the following year. During berry development and ripening, Buttrose, (1966) described a preferential partitioning of carbohydrates to the fruit at the expense of the leaves, shoots, roots and trunk. This corresponds with a local industry view that, for adequate ripening, shoot growth should cease at veraison. Miller *et al* (1993) suggested the relationship between low carbohydrate reserves in overwintering wood and reduced fruit yield in the following year. In Tasmania, leaves are generally falling as fruit is being picked, and if this preferential allocation of photosynthate persists up to harvest, the vine has limited capacity to build reserves in late summer and autumn.

An increase in turgor, the water potential gradient for water uptake is the driving force for berry cell expansion (Lockhart, 1965). The accumulation of sugars during the latter phases of berry development leads to a major decrease in osmotic potential of the berry, thus there is an increase in the driving force for water uptake and a corresponding increase in berry size.

Experimental Approach

Overall, the physiological and morphological changes involved in flowering, fruitset and berry development are well understood however, environmental and management influences have not been widely studied and industry representatives in Australia have raised concerns about the lack of ability to predict or obtain target yields (Martin *et al.*, 2000). For warmer climate regions there is concern about yield forecasting, and in the cooler Tasmanian region, there is a more immediate concern about how to manage the various components to obtain a yield somewhere near the target. To date, there is no local evidence to indicate whether bunch size or number drive the variation, or whether conditions at fruitset, flower initiation, or inter year factors are the main influences.

In this initial component of the study, vineyard yield and available weather data were analysed to identify local factors that might account for yield variability. As the data set contained yield figures only, it was necessary to use existing or extrapolated information on vine phenology to estimate the timing of key developmental stages in order to assess the weather influences on components of yield.

Materials and Methods

Relationships between yield and weather at the critical times in crop development were examined using yield data from the Tasmanian Licensing Board and seasonal weather data from the Australian Bureau of Meteorology.

Data collection

Fruit yield data for Pinot Noir and Chardonnay were obtained from the Tasmanian Licensing Board. Records commenced in 1990 and information was collated through to the 2000 vintage. A change in recording after vintage 2000 limited access to useful records to the 11 year period from 1990 to 2000. Records including address, planted hectares and yield per hectare, were available for every registered vineyard in Tasmania. Data contained no information on the age of bearing vines, vineyard structure, or management practices. Data had also been collated into state-wide and regional means for each year but contained no information on site to site variability.

Critical periods of crop development, based on the main determinants of yield, were taken as follows: initiation and initial floral differentiation (November in the year preceding), bud burst (September), flowering and fruitset (December/January) and fruit maturation (March/April). Exact timing for these events was not known for Tasmania, and dates were based on discussion with Tasmanian grape growers along with the cycle described by Coombe and Dry (2000) for mainland Australia. Data for rain days, total rainfall, mean daily maximum temperature, mean daily minimum temperature and the mean daily sunshine hours for the period 1988 to 2000 was obtained from the Australian Bureau of Meteorology stations at Hobart and

Launceston Airport and Grove Research Station, nearest three viticultural regions
Coal River Valley, Huon Valley and the North.

Data handling and analysis

For the State as a whole, the four viticultural regions and for selected vineyards, mean yield for Pinot Noir and Chardonnay over the 11 year period was calculated. These data were then used in regressions between vineyards or varieties and between yield and weather factors.

Multiple regression models (SPSS) were applied using the mean state yields and mean regional yields plotted against mean daily sunshine hours during hypothesised initiation (November) and at budburst the following year, and rainfall and temperature at flowering.

To reduce the impact of differences in vine age and management practices between properties, regressions of climate factors against annual mean yield were plotted for six vineyards selected because of relatively stable planted area and management during the 11 year period. These vineyards were also selected because each showed a positive correlation between yields for Chardonnay and Pinot Noir, suggesting consistent management throughout the period. Data for critical times in development (and yield the previous year) were plotted against yield in a stepwise regression procedure using SPSS. Data for each site are presented as significant ($P < 0.05$) multiple regression equations using the variables making a substantial contribution to the model.

Vine nutrition

The two vineyards used in the final stage of this study had been managed by the same personnel for the 11 years of yield record, and although no specific records of soil management, pruning or pest and disease control were available major changes in management seemed unlikely. To evaluate effectiveness of the fertiliser program leaf samples were taken in 2004 to check levels of the main nutrient elements likely to influence fruit set and yield. A bulk sample of the most recently expanded leaves was selected at random from different vines in a selected row near the middle of the main Pinot Noir blocks of each of the two sites. The total acid extractable phosphorous, and boron and molybdenum, were measured using ICP methods. Boron was measured using ICP-OES and molybdenum was measured using ICP-MS. Measurements were undertaken by the Central Science Laboratory, University of Tasmania.

Results

Yield patterns

There was no regular pattern in the mean state yields for the period of 1990 to 2000. Yields were considerably variable in the three regions across the period (North East: mean yield = 6.28 and standard deviation = 2.43, South: mean yield = 6.63 and standard deviation = 3.09, Tamar: mean yield = 6.85 and standard deviation = 2.75, State: mean yield = 6.05 and standard deviation = 1.79). Regional mean yields were all similar, but there were no significant regressions between regions. However in years in which low yields were evident, (1992, 1994, 1997 and 2000) the trend was

consistent across regions (Figure 2.2). Although there was some consistency in the trend for high yielding years, with 1995 and 1998 clearly showing higher yields, overall the relationships were weak.

The preliminary analysis of mean yield across the state (see Figure 2.2 for regional data) produced no significant correlations with weather data ($P>0.05$) for estimated critical periods in development.

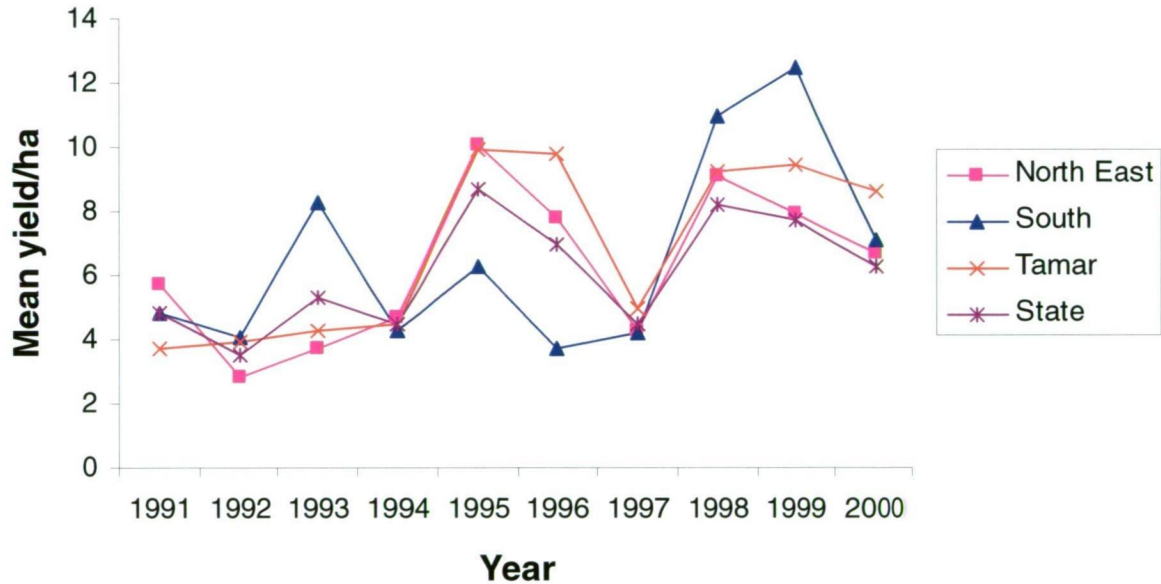


Figure 2.2 Mean yield of Pinot Noir (tonnes per hectare of grapes) for Tasmania from 1990-2000 per vineyard. Data are presented as State wide, and regional basis; North East; Tamar and South.

The yield data for both Pinot Noir and Chardonnay for six selected vineyards (Figure 2.3 and 2.4) exhibited a similar level of seasonal variability (Pinot Noir 1 tonne/hectare to 15 tonnes per hectare, standard deviation = 2.13; Chardonnay 1 tonne/hectare to 14 tonnes/hectare, standard deviation = 1.74) for all vineyards over the period of 1990-2000.

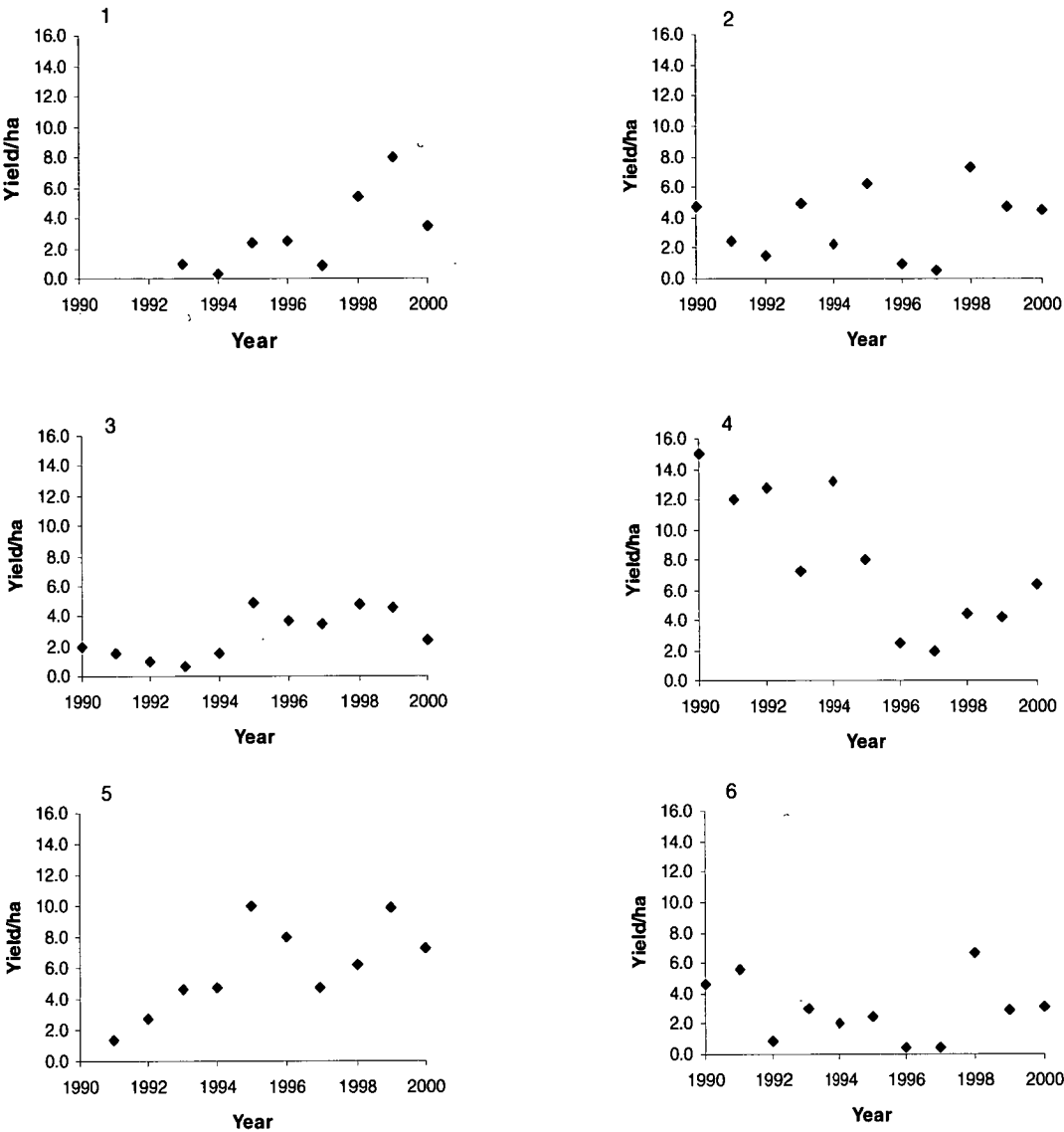


Figure 2.3 Annual mean yields in tonnes/hectare of Pinot Noir from 1990-2000 at six vineyards

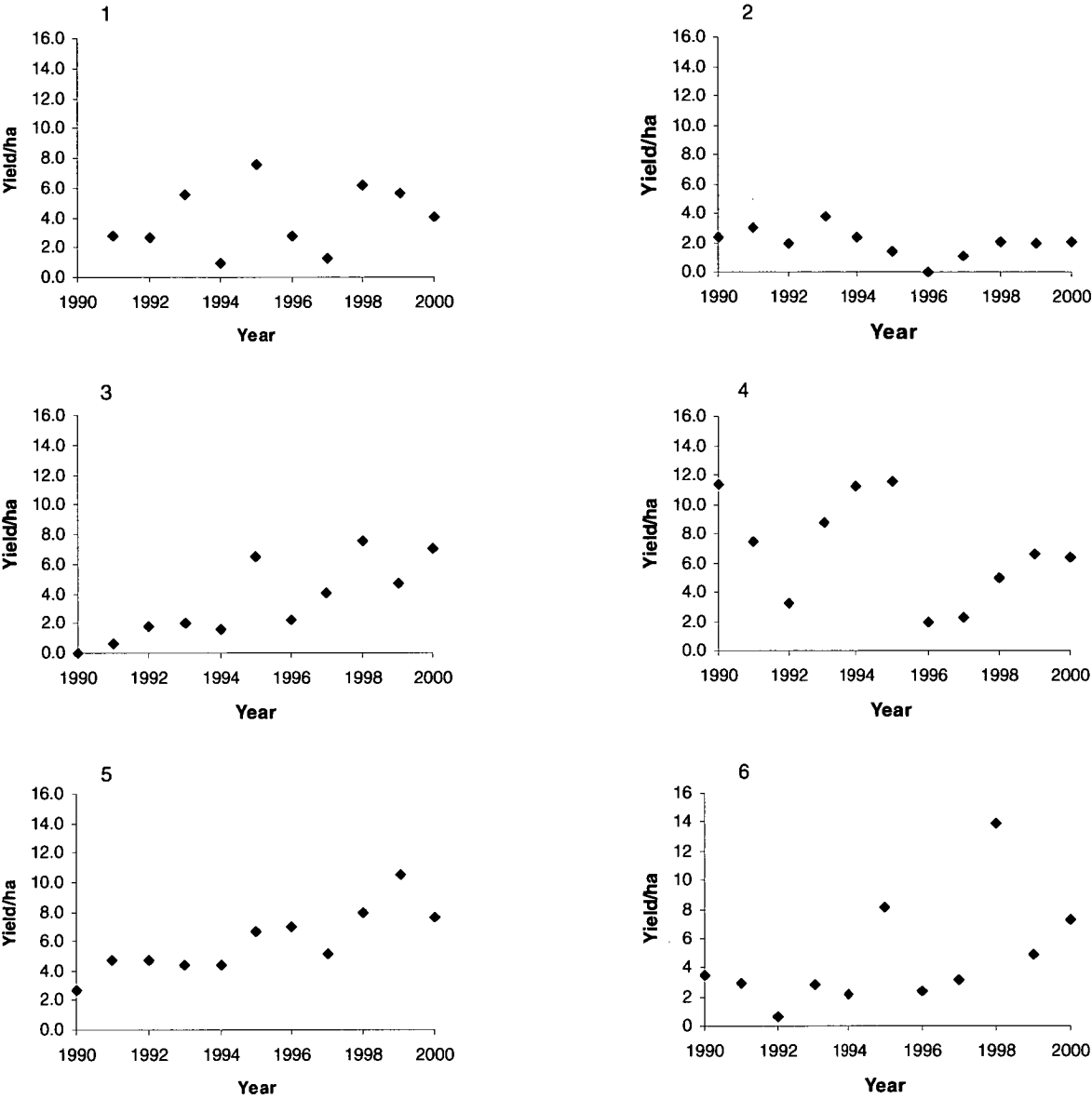


Figure 2.4 Annual mean yields in tonnes/hectare for Chardonnay from 1990-2000 at the same six vineyards.

Three (site 1, 2 and 6) of the six vineyards showed significant positive regressions between annual mean yield and either sunshine hours or temperature at flowering as shown in Table 2.1.

Table 2.1 Correlations between Pinot Noir yield and either mean maximum temperature at flowering or mean number of sunshine hours at flowering,. P= p value, r=Pearsons correlation coefficient.

Region	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
	South	South	North	North	North	South
	P r	P r	P r	P r	P r	P r
Temperature at flowering	0.047 0.61	0.084 0.57	0.460 0.24	0.900 0.17	0.900 0.17	0.048 0.19
Sunshine hours at flowering	0.117 0.64	0.048 0.19	0.150 0.47	0.340 0.32	0.580 0.20	0.020 0.69

For the site with the highest mean daily temperature, site 6, a multiple regression model of annual mean yield against mean maximum daily temperature at flowering, and average sunshine hours at flowering, had an r^2 of 0.90 with the following equation:

Yield= -53.34 + 2.35 (temperature at flowering) + 1.28 (sunshine hours at flowering)

Adding fruitset conditions, temperature and either total rainfall or mean rain days resulted in only a 2% increase in the explained variability ($p < 0.05$). At site 2 the equation:

$$\text{yield} = -10.00 + 1.68 (\text{sunshine hours at flowering})$$

accounted for 63% of the variability, adding rainfall increased the variability explained by the model to 76% with the following equation

$$\text{yield} = -8.01 + 1.43 (\text{sunshine hours at flowering}) - 0.18 (\text{rainfall at fruitset}).$$

Yield in the previous season had no effect on current season yield for either site.

Annual mean maximum temperature at flowering and annual mean yield at site 6 is shown in figure 2.5. For site 2, the relationship between annual mean sunshine hours at flowering and yield, and rainfall at and yield are shown in figures 2.6 and 2.7 respectively.

Weather data at flowering used for analysis for all six sites is shown in Appendix 1.

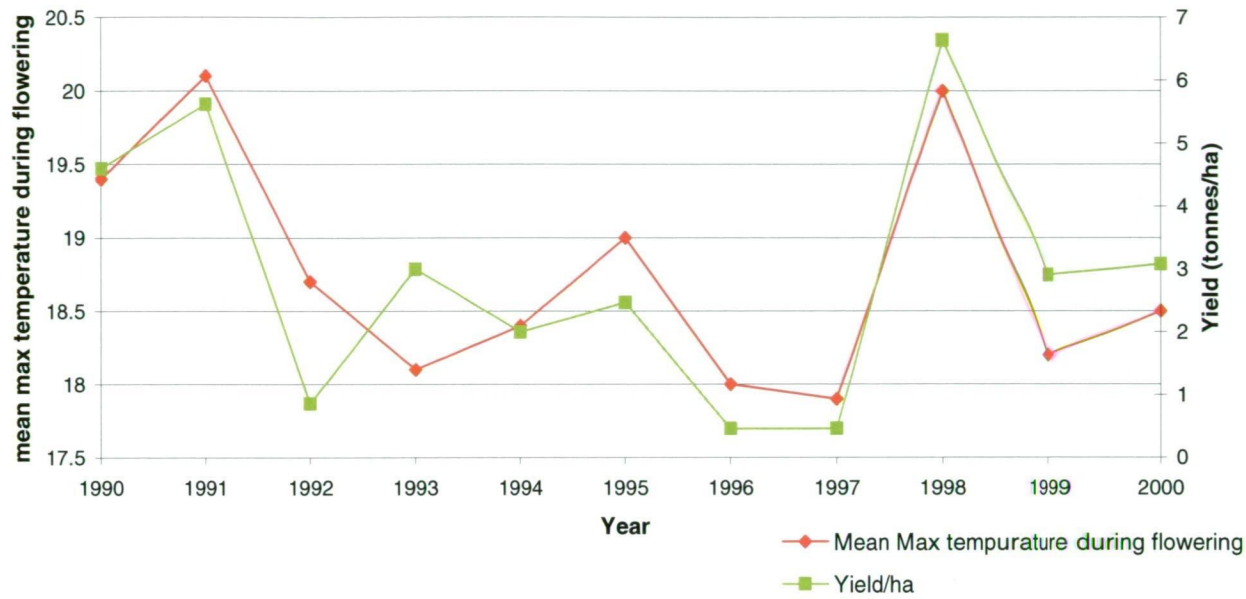


Figure 2.5 Temperature at flowering and the annual mean yield of Pinot Noir at Site 6.

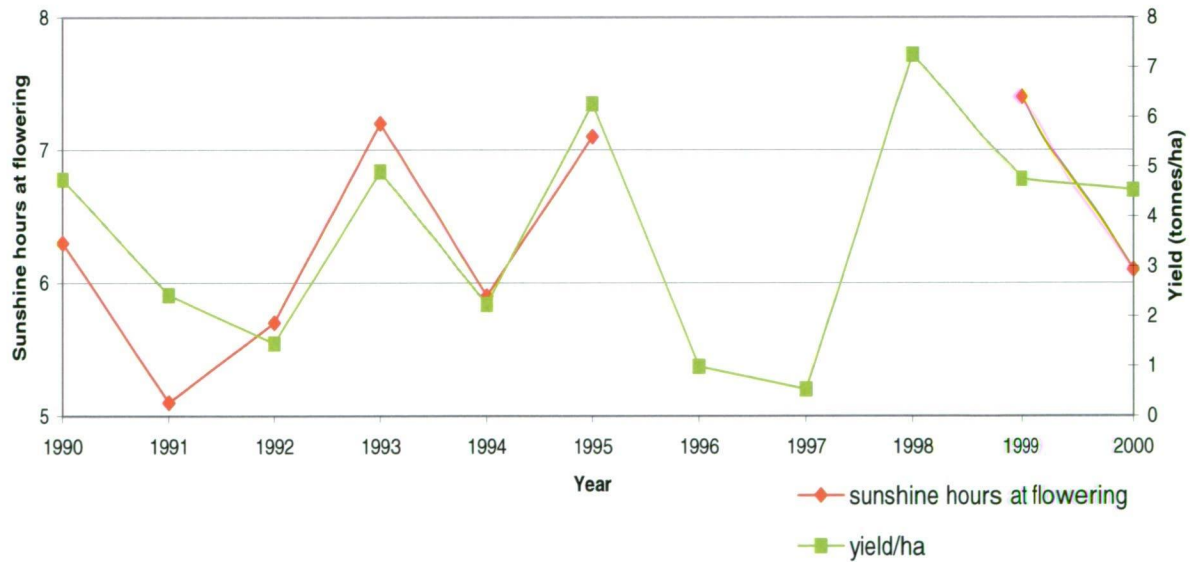


Figure 2.6 Sunshine hours at flowering and the annual mean yield of Pinot Noir at Site 2. (Note three data points were missing from the Bureau of Meteorology data set).

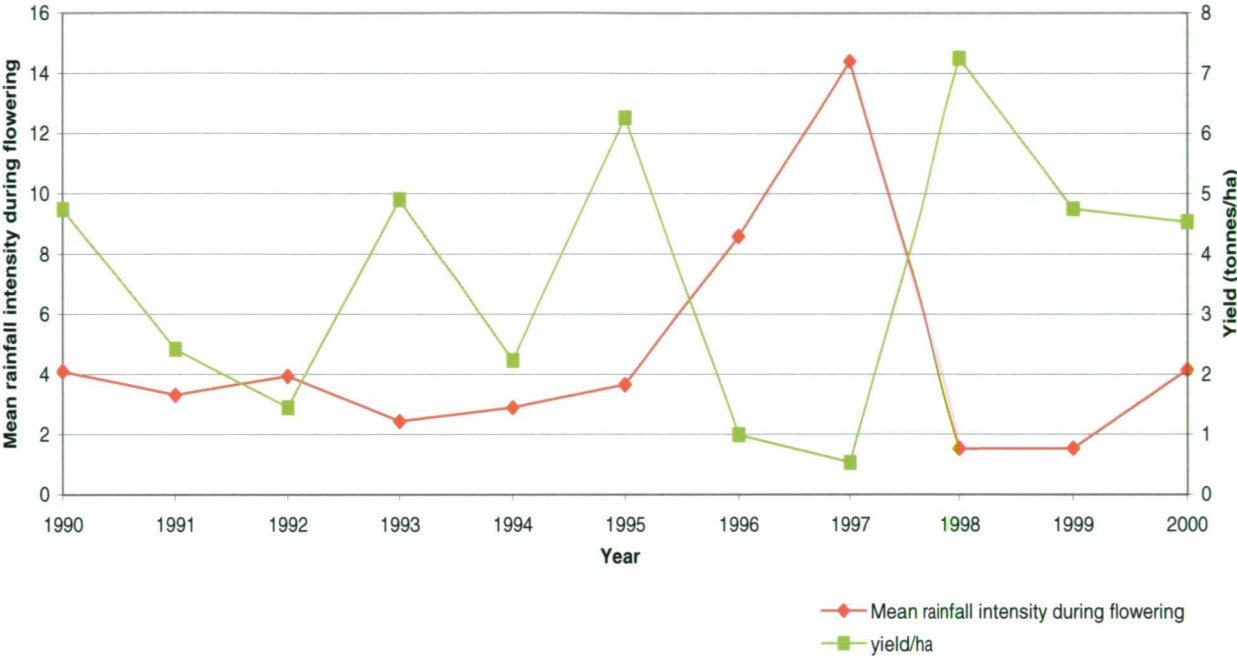


Figure 2.7 Rainfall at flowering and the annual mean yield of Pinot Noir at site 2.

Discussion

Analysis of industry wide yield data confirmed that yield variability is a major problem for Tasmanian vineyards. At one vineyard, yield varied from 2 tonnes/hectare to 15 tonnes/hectare for Pinot Noir, with a similar range for Chardonnay. Whilst patterns for regional means were less extreme, there was a fluctuation from 4t/ha to 12t/ha over a three year period for one vineyard in the southern region. Typically mean yields varied within a range from about 4t/ha to 8t/ha across each of the four regions.

In spite of the fluctuations from season to season, a general improvement in fruit yield was apparent in each of the regions as well as on a state basis (figure 2.2). This

could be a reflection of increasing vine age in the larger vineyards which dominate the production record. Improvements in vine management could also be responsible for the steady increase in fruit yield. It was notable however that the overall increase in yield does not appear to be accompanied by any improvement in stability.

The anomalous trend of decreasing yields for Pinot Noir at site 4 can be explained. This site has employed management strategies to intentionally decrease yield in accordance with the belief that lower yields achieve higher wine quality. This strategy is used primarily on the varieties Pinot Noir and Cabernet Sauvignon as these are the vineyard's flagship labels, but not on the Chardonnay crop (figures 2.2 and 2.3).

Of the selected vineyards only two showed patterns consistent with each other and between varieties. The two vineyards that displayed these trends were amongst the oldest in the state and there had been no increase in the planted area or replanting at either site. The number of vineyards available for this analysis was small with only 5 commercial vineyards in the region having the 11-year yield record. Most of the established vineyards in this region are within 20 km of the two weather stations used in the analysis. In the northern region all vineyards with the full 11-year yield history were at greater distance from weather stations (60 + km).

The results confirm that the issue of yield variability is real and of a magnitude sufficient to threaten economic viability of vineyards. The fact that a similar level of variability was evident for Chardonnay and Pinot Noir, confirmed that there is

nothing peculiar about Pinot Noir and that it doesn't behave differently to another cool climate variety.

The regional mean data provides some insight into the possible effects on yield. Industry comments on the poor southern vintage of 1996 note poor fruitset conditions in southern Tasmania, which is reflected in the very low yields of sites 2, 4 and 6 for both Pinot Noir (figure 2.3) and Chardonnay (figure 2.4) (Farquhar, 2004). Other regions and sites did not demonstrate such low yields in that year, consistent with the fact that the weather patterns were predominantly southerly and south-easterly (Farquhar, 2004) with little impact on regions nearer the northern coast. Yields in both regions and at sites 2, 4 and 6 were also low in the following year of 1997, which could reflect the poor floral initiation conditions during 1995. The high yields of vintage 1998 at sites 2 and 6, were possible due to favourable pollination conditions in 1997 leading to a high percent fruitset.

When state-wide and regional yields and weather data for critical crop development periods were correlated no significant relationships were apparent. The selection of the six vineyards based on relatively stable management for further analysis was undertaken to reduce the impact of management and vine age as sources of variability. There was no overall agreement between yield patterns in this reduced data set suggesting that, if there is a weather effect, it was overridden by local management factors, or that other local climate issues (frost for example) which could not be extracted from available weather records in this study may have had a significant impact. Nevertheless significant relationships between weather and Pinot

Noir yields were identified in three of the selected vineyards. Of the sites that gave no relationship between yield and weather factors, all were located more than 30km from the nearest weather station. It is thus possible that the weather data used in the analysis may not have accurately reflected conditions at the three vineyards, further contributing to the lack of significant correlations in the remaining three sites. Two properties (sites 2 and 6) had been producing Pinot Noir from an unchanged area, with relatively constant management approaches, throughout the 11 year period. These two properties and a third (site 1) gave significant regressions between spring weather conditions as either sunshine hours or temperature and yield.

The contribution of conditions at flowering to yield may relate to fruitset and indicate that low temperature and rain during the three week flowering period contributed to reduced bunch size. Bunch size has been identified as the second most important factor in yield prediction models (Martin *et al*, 2000) in a climate classified as cool by the authors but generally warmer than Tasmania. A further difficulty in evaluating any correlation between fruitset with weather from historical data is the lack of any precise information on the time of flowering. As already noted (May, 2004) cap fall can last for a period of about three weeks in many vineyards, but the start appears to vary markedly within a locality. Therefore the lack of major effect of climatic conditions during flowering at sites 1, 3 and 5 may simply be related to the precise timing of flowering.

It would also be necessary to redefine the precise timing of the other major reproductive events to align with the climate data at that stage. As noted, the timing of the events in this preliminary study were determined after communication with local viticulturists, and assumed constant from year to year.

The results presented in this chapter highlight the small amount of evidence that weather has an effect on yield. Previous work has found that weather conditions, especially in the spring, directly influenced the fruitfulness of the buds produced that year (Antcliff and Webster, 1955). Winkler *et al* (1974) suggested that in the regions where vines are generally grown, it seems unlikely that temperature encountered during fruit-bud formation would have appreciable direct effects.

The lack of a significant correlation between weather conditions during inflorescence initiation and final yield highlighted in this study, questions the importance of bunch number as the primary determinant of yield. Given that bunch number has been previously identified as the primary determinant of yield in cool climates (Martin *et al.*, 2000) it was hypothesised that weather conditions during the period of inflorescence initiation would influence yield. The fact that no such relationship was identified may be due to the widely used practise of bunch thinning to achieve target yield employed by growers in the majority of vineyards throughout Tasmania (Farquhar, 2004). Since the number of bunches per vine is thinned to a standard level each season, the variation in yield between seasons would be fully explained by bunch size, either in the form of berry number or berry weight. Both of these

parameters are influenced by pollination and fruitset, which in turn are strongly influenced by weather during flowering and fruit development (May, 2004).

It was not possible to get a retrospective measure of the nutrient status of the vines over the time in question, however it was considered valuable to have some idea of whether levels of previously identified nutrients of importance in flowering and fruitset may have been limiting. The fertiliser regime employed at vineyard sites 2 and 6 remained constant during the period of yield analysis and up to the present. Leaf tissue composition was assessed in 2004 and found to be in the adequate range (Weir and Cresswell, 1993) for boron, phosphorous and molybdenum (Appendix 2), suggesting that levels were probably adequate for the period in question.

Further detailed research is necessary for these relationships to be analysed fully, however it is possible that sunshine hours, temperature and rainfall intensity at flowering and fruitset may have an impact on final yield. As there is no obvious way of directly influencing the weather, growers need to adopt management strategies to manage the potential variability induced by weather conditions at flowering. Given that other researchers have shown that bunch number is the main component of final yield (Martin *et al.*, 2000) possible management approaches to manipulate bunch number became the main focus of this study. Also, in view of uncertainties about the process of flowering and fertilisation, and a possible link with weather conditions at flowering shown here, some attention was also paid to aspects of flowering and vineyard management during anthesis.

Testing the model

After the completion of the analysis period, the weather data for the vintages 2001, 2002 and 2003 (Appendix 1) for the two vineyards with significant positive regressions between annual mean yield and either sunshine hours or temperature at flowering was entered into the respective equations, derived above, and the resulting predicted means were compared with the actual means provided by the growers.

For the vintage of 2001, the equation for Site 2 gave a predicted yield equivalent to 99% of the actual yield and the equation for Site 6 accounted for 95% of the actual. In 2002 the equations gave a poor yield prediction, with the model predicting yields to be markedly lower than the actual yields at both sites. In 2003 the equation at Site 2 underestimated yield by 48% and at Site 6, yield was overestimated by 17%.

For both 2001 and 2003 yield predictions using the two models were well within the overall range in variability. There is no clear reason for the marginal accuracy in 2003 for Site 2. The poor result for both sites in 2002 may be explained by the very low sunshine hours at flowering experienced at both sites, well outside the range included in the model.

Overall, the results for the three years reflect the industry conditions for the three years, with Farquhar (2004) noting the unusually low yields industry wide in 2002. As two of the three years were included in the three years of field trials reported in this thesis, trial results reflect these seasonal influences in yield.

Chapter 3 – Influences on yield during flowering - pollination and fruitset

Introduction

As discussed in the previous chapter, temperature and sunshine during flowering in Tasmania were found to explain much of the variation in yield at several vineyards, but there were no obvious weather / yield relationships in others. Individual vineyard management practices, may therefore be having a significant effect on yield. Given that flowering to fruitset has been identified as a key phase influencing yield (Dunn, 2003), this chapter examines this part of the reproductive cycle and associated management practices that may influence yield.

A review of the literature on flowering in vines revealed a wealth of information about flowering biology but a lack of information on pollination fertilisation, and fruitset. Uncertainty about the timing of pollination and fertilisation and methods of pollen transfer were noted by Winkler *et al* in (1974) and little has changed through to the present (May, 2004). Although there is little opportunity to alter or manage direct influences of weather on fruitset, a significant management practice during the three to four week flowering period is the application of one or sometimes two fungicide sprays for control of bunch rot (*Botrytis cinerea*) and Powdery and Downy mildew (*Uncinula necator* and *Plasmopara viticola* respectively).

Flowering in the grapevine

V. vinifera flowers are small and in most cases are green. The complete flower consists of 5 small sepals joined at the base, a calyptra of 5 petals fused at the tip and 5 stamens each with a double lobed anther. The calyptra ejects during flowering to expose the reproductive organs of the flower, and the process is generally termed “cap fall”. In the centre is a pistil with a short style and a thick lobed stigma. The ovary is divided into 2 parts, each containing 2 ovules with individual embryo sacs (Lavee, 1985), to give a potential four seeds per berry.

Most grapevine cultivars are highly floriferous and inflorescences are formed on shoots that arise from most nodes along the cane. Each inflorescence may contain up to a thousand flowers. Of these flowers, 70-80% normally fail to develop into mature fruits, dehydrate and abscise.

The term ‘fruitset’ is used, as in most other commercial perennial fruit crops, to describe the transformation of flowers into fruits (Mullins, 1992). In practical terms, because of the number of flowers in each cluster, there are some difficulties in making an objective assessment of fruitset. In tree fruit, the figure is usually given as number of fruit per 100 blossom clusters (Coombe, 1989). Similarly in grapevines, fruitset is taken as berries per bunch, usually with no count of flower number (May, 1992). In a system where bunch number is largely determined by pruning and berries per bunch are much higher and potentially more variable than in tree fruits, this appears to be an inadequate measure of fruitset. To overcome this difficulty several methods have been devised to make flower counts (May, 2004), including

electronic counting devices, counting flowers on photographs and a combination of photography and mathematical modelling. Despite berries per bunch being a poor measure of fruitset, many studies have linked very low berry number per bunch with a number of factors considered to influence fruitset. Nevertheless, such results appear to have been obtained only with large changes in bunch size and use of berry number alone fails to give any indication of whether “set” was influenced at initiation, during differentiation or after fertilisation.

Poor fruitset has been associated with inadequate levels of pollen at the style, failure of fertilisation, embryo abortion or abnormal flower development (Srinivasan and Mullins, 1981; Sedgley, 1989; Ebadi *et al.*, 1995). Air temperatures below certain limits have been reported to affect fruitset detrimentally and in a major way through reduced pollination and fertilisation (May, 2004). Conversely, high temperatures are also damaging, as percent fruitset of Pinot Noir vines was significantly lower at 35°C and 40°C than at 25°C daytime temperatures (Kliewer, 1977). Rain during the flowering (anthesis) period and low nitrogen levels during inflorescence initiation also have a detrimental affect on fruitset as do deficiencies of minor elements such as boron, zinc and molybdenum (May, 2004).

For successful fruitset to occur, a sequence of events including transfer of pollen from anther to stigma, pollen germination, tube growth to the ovary and finally successful migration and fusion of the gametes must take place. Pollen transfer is generally thought to occur only when flowers open and the opening of flowers (cap fall) usually occurs early in the morning or from 2 to 4 in the afternoon (Pratt, 1971).

Staudt (1999), also reported that in *Vitis vinifera* opening of flowers within a single inflorescence followed a periodic 24-hour cycle. However, in this study, flowers opened at a definite time of the day, following a rhythm that started at about 5am and ended at about 12am, with few opening in the afternoon. Within the cycle the short time flowers were open was increased by lower temperatures and decreased by higher temperatures. Low temperatures have also been reported to reduce the number of open anthers or delay opening (Winkler *et al.*, 1974).

There is considerable debate and uncertainty around the issues of pollen transfer, and cross and self-pollination. In a 1971 review, Pratt reported that some studies state that pollen dispersal is primarily by wind (Viala and Pechoutre, 1934) while others suggest insects play a role (Armstrong, 1936) and many researchers have suggested self-pollination ((Sartorius, 1926; Suessenguth, 1953; Winkler *et al.*, 1974); cited in Pratt, 1971). Mullins (1992) reinforced the uncertainty surrounding the mode of pollen transfer, stating that the structure of the flower is not suggestive of wind-pollination. It was concluded that, while there is a lack of consensus in the literature as to whether the genus is self-pollinated, cross-pollinated or both, the common commercial *vinifera* cultivars may be selfed.

It has been reported that Pinot Noir is one of two varieties of *Vitis vinifera* L., which are hermaphroditic and self-compatible and anthesis has been shown to occur before or during the flower opening process (Staudt, 1999). By extrapolating from previously determined pollen tube extension rates and observed extension of the pollen tube after cap fall, Staudt (1999) concluded that 16-18% of flowers were

pollinated before flower opening and the growth of pollen tubes had already started by cap fall. While Staudt's work did not preclude wind pollination, it cast considerable doubt on the accepted theories of pollination especially for Pinot Noir, providing strong evidence to indicate self pollination. Further, the timing of fertilisation 2 or 3 days after pollination as suggested by Pratt (1971) is incorrect for Pinot Noir and that unless pollen tube growth is delayed by low temperature or some other reason, fertilisation takes place at around the time of cap fall. Staudt (1999) stated that at average temperatures, pollen grains need only 0.5 h on the stigma to become ready for germination. Staudt, (1982) also found that the time to fertilisation was affected by temperature and between 25 and 28°C some pollen tubes reached the nucleus after 12 hours, whereas at 15°C, the time for pollen tubes to reach the nucleus had extended to 48 hours.

Temperature influences the growth of the pollen tube and also the longevity of the ovules, with the optimum temperature for pollen tube growth being greater than that for the longevity of ovules (Sedgley, 1989). Consequently a decrease in effective pollination periods during warm weather may reflect ovule longevity rather than any direct effect on pollen. However high temperatures have also been reported to impact on pollen viability and vigor (Shivanna *et al.*, 1991; Aronne, 1999; Aloni *et al.*, 2001).

Pollination as influenced by vineyard practices

There are two key management practices carried out during flowering in the vineyard; (1) Shoot tipping and tucking, and (2) spraying for diseases. Both practices are routine operations generally scheduled at around the time flowers open, however little is known about impacts on pollination, and subsequent fruit yield.

Shoot tipping during flowering has been shown to promote fruitset (Coombe, 1962). Total reliance on reserve carbohydrates for shoot growth ceases when the first leaves reach half their final size (Mandel *et al.*, 2001). The newly formed assimilates move strongly towards the shoot apex, which is a stronger sink than the young inflorescences. Removing the shoot tip at the time of anthesis, when half the caps have fallen was reported to promote fruitset (Coombe, (1959).

In Tasmania fungicide applications in grapevines during full bloom are common practice, even though, for other crops, it has been demonstrated that the active ingredients and other components of commercial formulations can adversely affect growth of pollen tubes (Gentile *et al.*, 1978; Marcucci and Filiti, 1984; Elezaby and Haseeb, 1995; He *et al.*, 1996; Pavlik and Jandurova, 2000; Dag *et al.*, 2001; Kopcke *et al.*, 2002). A negative effect of fungicides on the *in vitro* germination of pollen of several species has also been shown (Eaton, 1961; Gentile *et al.*, 1978; Marcucci and Filiti, 1984; He *et al.*, 1996; Nikolov *et al.*, 1999). In these studies fungicides incorporated into the media, or sprayed on the surface at rates up to those recommended for disease control, reduced pollen germination and pollen tube growth at concentrations considerably lower than those commercially recommended.

Working with grapes Nikolov *et al* (1999) found that a selection of fungicides used to control *Botrytis cinerea* and other common diseases had varied effects on pollen germination and pollen tube growth of the varieties Bolgar and Cabernet Sauvignon. In Cabernet Sauvignon chlorothalonil reduced pollen germination by 10 percent and cyprodinil by 13 percent. Both significantly reduced pollen tube length in the same variety.

This chapter reports two studies. The first was aimed at confirming Staudt's (1999) observations on pollination in Pinot Noir, as a basis for improved understanding of the vulnerability of fruitset to weather conditions. The second group of experiments studied the potential effects of three commonly used *Botrytis* fungicides in cool climate viticulture on the *in vitro* and *in vivo* germination of pollen of Pinot Noir and effects on pollination / fertilisation in the field. In the local industry, commercial recommendations for application of fungicides for control of *Botrytis cinerea* during flowering vary from fungicide to fungicide, but application at early flowering (5% cap fall) and/or late (at 80% cap fall) are usual. This represents a flowering period of 3-4 weeks in most seasons.

Materials and Methods

Method of pollination

Investigations were carried out in the 2002/2003 flowering season on Pinot Noir clone 2051, in a commercial vineyard in southern Tasmania. Weather data from the

nearest Australian Bureau of Meteorology Station at Grove Horticultural Research Station was collated for the flowering and fruitset periods (November and December 2002 and January 2003).

Flower Sampling

Inflorescences were sampled for assessment of key morphological features associated with flower development. Flowers were sampled at different stages of the flowering cycle, ranging from fully closed with the calyptra intact, through to flowers that had been open for several days (tagged at cap fall). Stages of flowering were; pre-cap fall, cap beginning to roll back, calyptra fully ejected, calyptra ejected and stigma beginning to brown, calyptra ejected and stigma dry. At each stage, inflorescences were removed from the vines in early morning and kept in a pre-cooled sealed vial in an insulated container for transport to the laboratory (approximately 30 minutes), where they were refrigerated at 4°C for a maximum of 4 hours before examination.

Scanning Electron Microscopy

Flowers were examined using an ElectroScan ESEM2020 Environmental Scanning Electron Microscope (ESEM) to record changes in stigma surface and the presence or absence of pollen grains. The instrument was operated at an accelerating voltage of 15kV in environmental mode, using water vapour as the imaging medium. Secondary electron images were acquired using the proprietary ElectroScan Gaseous Secondary Electron Detector (GSED) using water vapour as the imaging gas at a nominal pressure of 5 Torr. To obtain images of the stigma surface prior to natural

cap fall, the cap was carefully removed using fine forceps, just prior to placing the flower in the ESEM chamber. Five flowers were examined at each stage.

Light Microscopy

The change in appearance of anthers during dehiscence was recorded photographically using a dissecting microscope with a camera adapter. Caps were carefully removed from mature flowers for photographs. Excised stigmas were mounted in a drop of basic fuchsin gel and examined under a light microscope for counts of pollen deposition in both opened and capped flowers. Using this technique, grapevine pollen stained red against a background of unstained stigmatic tissue. The basic fuchsin gel was prepared, after the method of (Spurr, 2003). Forty grams of hydrated gelatin was melted in a microwave oven and mixed with 60mL of glycerol. Basic fuchsin powder was gradually added to the mixture until a transparent mid red gel matching a colour standard determined in preliminary trials to differentiate pollen and stigmatic tissue of grape flowers was obtained. The resulting mixture was filtered through glass wool and stored at 4°C.

Pollen Viability

Bulk pollen samples were collected from flowers with intact caps, at cap fall and from flowers, which had been open for some time, but showing no evidence of fruit development. Flowers (with caps removed) were shaken over a 1.5ml Eppendorf tube, and the collected pollen desiccated over freshly dehydrated silica gel in a small sealed container. After dehydration the tubes were sealed and stored at -80°C.

Samples (150-300 grains) were taken from the stored bulked pollen and re-hydrated

by placing open tubes containing pollen over a water bath at 35°C for 60 minutes.

Viability was determined using the fluorochromatic reaction test (FCR) of Shivanna and Rangaswamy (1992).

In order to determine the most appropriate concentration of fluorescein diacetate solution (FDA) to maximise pollen viability for *in vitro* studies on pollen germination, a number of sucrose concentrations were tested on fresh pollen. Stock of FDA 2mg/mL was prepared in acetone and stored at 4°C. The stock solution of FDA was added drop by drop to 2 mL of 5%, 10%, 20% and 30% sucrose solutions, until the mixture showed persistent turbidity. The mixture was used within 30 minutes of preparation to prevent the FDA from precipitating. A drop of this solution was placed on a microscope slide and a small number of pollen grains were suspended in it to ensure uniform distribution of the pollen in. A glass coverslip was lowered over the drop and the pollen grains that fluoresced brightly were counted under a fluorescence microscope. The preparation was observed immediately as the fluorescein eventually leached from the pollen grains (Shivanna and Rangaswamy, 1992). At least 250 pollen grains were counted in randomly selected fields under a fluorescence microscope (a Leica Leitz Model DM RBE). The highest pollen viability was obtained using the 10% sucrose solution (see Appendix 3), this concentration was used in all further studies.

Pollen Tube Growth

Both capped and naturally open flowers were collected for examination of pollen tube growth on stigmas. Stigmas were excised, placed on a slide, covered with a

drop of aniline blue stain, and a cover slip and left at room temperature for four hours. The aniline blue stain was made by dissolving 5.75g of K_3PO_4 and 0.125g of water-soluble aniline blue in 250ml of distilled water. The fluorescence microscope was used to view the mounts and the total number of pollen grains and the number with pollen tubes counted (Shivanna and Rangaswamy, 1992).

Selfed or Open Pollinated

Ten inflorescences developing mid-way along canes on separate cane pruned vines were selected at random in a commercial vineyard. Prior to cap fall, on 22 December 2002, five un-opened inflorescences were enclosed in small white waxed paper bags and sealed with cable ties around the base of the rachis (see Plate 3.1). This bagging arrangement is used in commercial forestry to eliminate any transfer of pollen into or out of the bag (Moncur, 1995; Patterson *et al.*, 2004). Five un-opened inflorescences were similarly selected, labelled and left uncovered. Sixteen days later on January 7, 2003, after fruit expansion had commenced, bags were removed and the number of expanding berries present and the total number of flowers (including abscised flowers retained in the bag) in all ten bunches were recorded. At commercial harvest each bunch was removed and bunch weight and berry number counted. Fruitset was calculated as the ratio of developing fruit to original flower number.



Plate 3.1- Bagging to exclude transfer of pollen into or out of the bag.

Fungicide toxicity

In vitro experiments

Excised anthers of cultivar Pinot Noir were collected from vines in a commercial vineyard in Southern Tasmania, desiccated and stored in Eppendorf tubes at -80°C until required.

Commercial formulations of three fungicides, at the concentrations listed in Table 3.1, were added to a liquid germination media containing 10% sucrose, 1mM boric acid and 1mM calcium nitrate. This method was adapted from Shivanna and

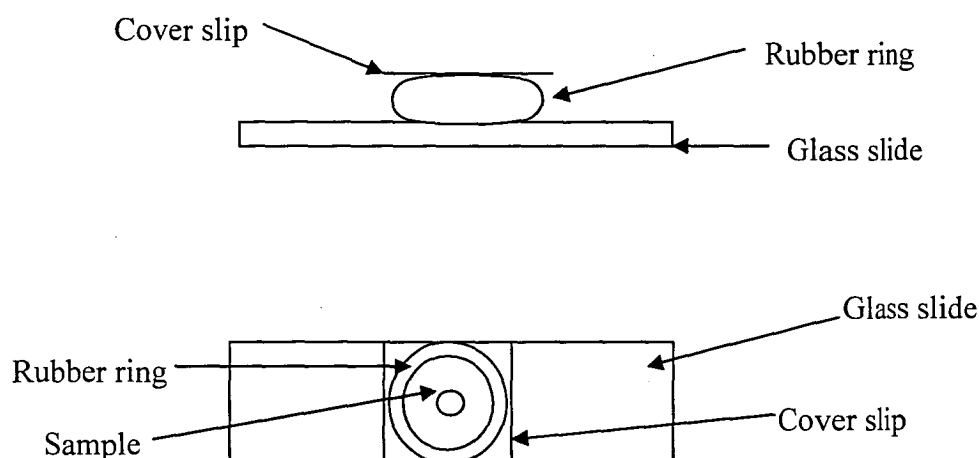
Rangaswamy (1992) with the optimum sucrose concentration determined as reported earlier. For each concentration of fungicides the pH was measured and buffered (if necessary) to the original pH of the nutrient solution.

Table 3.1: Fungicide concentrations of liquid pollen germination media containing three commercial botrytis fungicides.

Fungicide	Concentration			
	<i>Recommended Concentration</i>	<i>10% dilution of recommended</i>	<i>1% dilution of recommended</i>	<i>0.1% dilution of recommended</i>
Pyrimethanil	2mL / L	0.2mL / L	0.02mL / L	0.002mL / L
Cyprodinil + fludioxonil	0.8g / L	0.08g / L	0.008g / L	0.0008g / L
Chlorothalonil	1.6mL / L	0.16mL / L	0.016mL / L	0.0016mL / L

The hanging drop method described by Elezaby and Hasseeb (1995) was used for pollen germination. One drop of the nutrient media containing the fungicide was placed on a slide cover slip. Stored pollen was rehydrated for 30 minutes at 35°C over a water bath as described above, before testing. Three anthers from different flowers were gently stroked across the surface of the drop to deposit pollen. A rubber ring was attached to a slide with petroleum jelly and then more petroleum jelly was coated on the top of the ring. The cover-slip was then placed on the top of the ring, with the drop hanging in the centre of the ring, elevated above the slide (diagrams 3.1a and b).

Diagram 3.1a: Hanging drop method for germinating pollen in liquid media from side view (top) 3.1b: and from above (bottom). Petroleum jelly was used to seal the top and bottom of the rubber ring.



The slides were left for 10 hours in an incubator (Contherm Incubator 145MCP) set at a constant 20°C with 15 Watt fluorescence tubes, producing a measured photon flux density at slide level of approximately $40\mu\text{molm}^{-2}\text{s}^{-1}$, before germination level and tube length were measured. Pollen was recorded as germinated if the tube length was equal to grain diameter. Germination percentage was determined on a count of 450 grains and the length of 120 randomly selected pollen tubes was measured. Pollen tube form was also noted as being normal or malformed.

Statistical Analysis

The trial design was a three chemical by four-dilution rate factorial with an external control (no fungicide) in a randomised complete block design. For analysis of variance, dilution rates in which no germination occurred were deleted from the

calculation to give an analysed design of only two dilution rates in the factorial. The analysis was carried out on the general linear models package of SPSS, with Fischers LSD ($P=0.05$) values calculated after Steel and Torrie (1980), for comparison between treatment means. Percent germination data was subject to arcsine square root transformation for analysis of variance.

Field experiment

Pyrimethanil was applied at the recommended rate in the commercial vineyard that pollen for *in-vitro* trials had been collected from. Fifteen vines were chosen at random in a section of the vineyard and randomly assigned to three treatments in a five replicate by three spray treatment completely randomised design. Portable spray screens of plastic sheet were erected during spray application to protect trial vines from spray drift. The first treatment was sprayed at 5% cap fall, the second at 80% cap fall and the third was a control, not treated with fungicide during the flowering period. Treated vines were buffered with three additional sprayed vines on either side. Spray was applied to run-off using a hand operated sprayer and control vines were sprayed with water. For treated vines, a single bunch was chosen at random and tagged for fruit set counts. Flowers on each tagged bunch were counted before and after treatment, fruit numbers on the same bunch were counted at the pea-size stage and again at harvest. Fruit set was calculated as the proportion of flowers setting fruit. Seed counts were performed at harvest and seeds were tested for viability by the water floatation test of Wakana *et al* (2002). Trial design was completely random with three treatments and four replicates. Data transformation and analysis were as described above.

Results

Method of pollination

Weather Data

Monthly average maximum temperatures were 19.9°C for November, 21.5°C December and 25.0°C for January. Corresponding mean minimum temperatures were 7.4°C, 9.3°C, and 10.4°C respectively whilst average relative humidity for the three months was 66.6%, 61.4% and 60.5% respectively. Precipitation was 33mm for November, 13mm for December and 23mm for January.

Scanning Electron Microscopy

ESEM images showed that pollen was already present on the stigmas of capped flowers. Cells on the exposed surface of the stigma also appeared turgid at this stage. Pollen grains were slender and elongated, with a length of around 35µm and a mean length/diameter ratio of 2.3 (Plate 3.2a). Stigma surfaces of open flowers had a more flaccid appearance and pollen grains were less elongated in shape (Plate 3.2b and 3.2d), with a length of around 28µm and a length/diameter ratio of 1.4. Pollen grains appeared to be tricolpate and of medium size when contained within the anther (Plate 3.2c). There were no pollen tubes or evidence of pollen tube growth visible in ESEM images for flowers before or after cap fall.

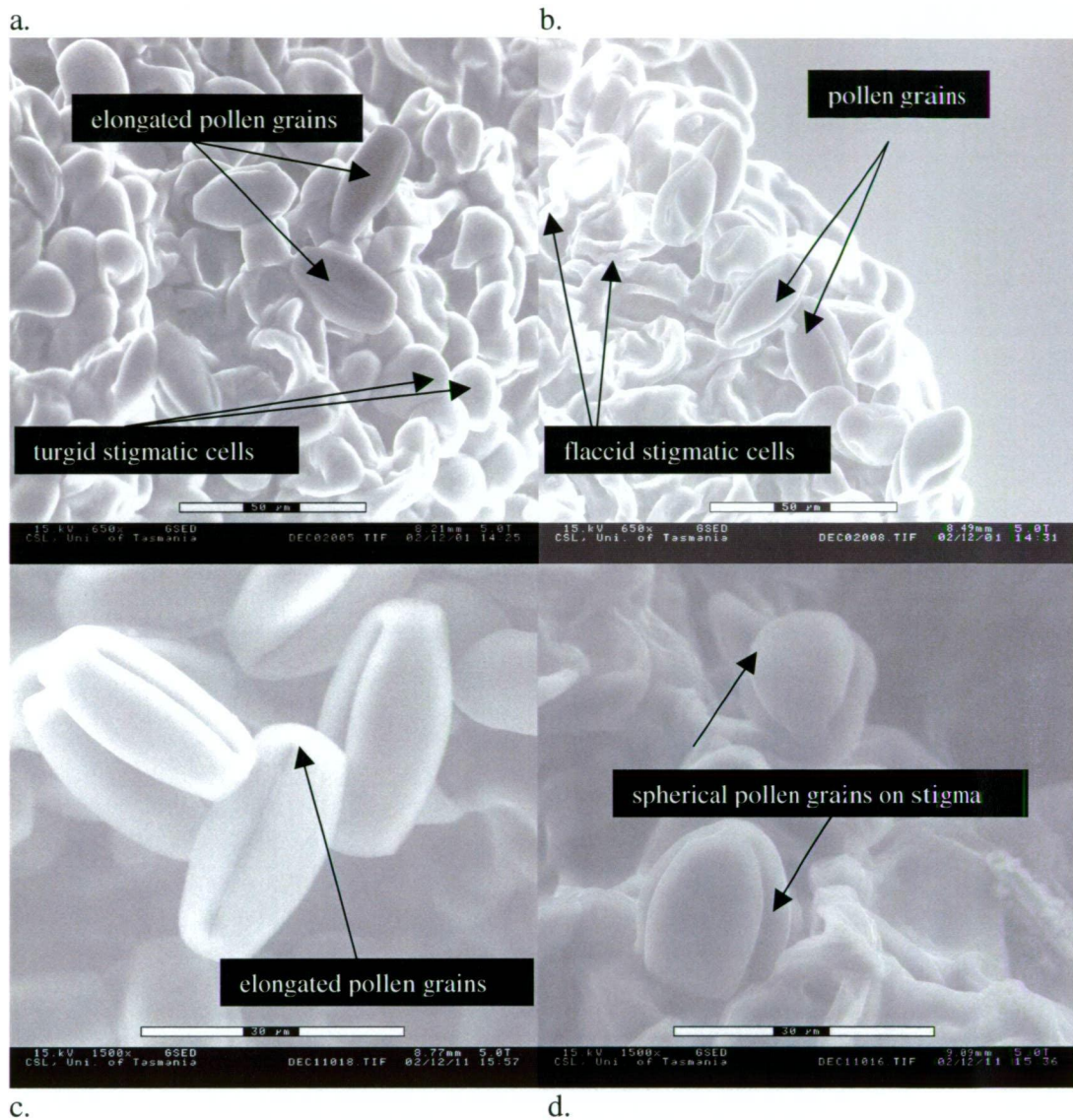


Plate 3.2 Electronmicrographs of flowers before and after cap fall showing:

- (a) the stigma surface of a capped flower with turgid stigmatic cells.
- (b) the stigma surface of an open flower, with flaccid stigmatic cells.
- (c) elongate pollen grains contained in an open anther of a capped flower.
- (d) pollen grains on the stigma of an open flower, where pollen grains are less elongate.

Anther Dehiscence

When closed, the anthers contained two lobes, each smooth in texture and plump in appearance. Opening of the lobes commenced with a gradual split along the line separating the two lobes. When the lobes were completely open, the structure folded back to give the appearance of two discs of pollen. This latter stage was already evident in closed flowers where the anthers were exposed by removal of the cap. The fuchsin stain technique confirmed pre-cap fall release of pollen shown by the presence of pollen grains on the stigma of capped flowers. The mean number of pollen grains present on the stigma surface after natural cap fall, 423.7 ($s = 39.2$), was far greater than the mean number present on unopened flowers examined by removal of the cap, 92.3 ($s = 15.3$).

Pollen Viability

Pollen viability tests showed no significant differences between capped flowers, flowers at cap fall and open flowers. Viability of pollen removed from capped flowers was 51.6% ($n = 155$ counted flowers), removed from flowers at cap fall 55.2% ($n = 324$) and from open flowers it was 57.0% ($n = 219$).

Pollen Tube Growth

The staining method gave definitive results without the need for clearing of the stigmatic tissue. In the thirty open flowers examined, each showed obvious pollen tube growth in the style (plate 3.3). Mean pollen tube length was 513 μ m ($s = 203$) with shorter pollen tubes around 350 μ m being found in recently opened flowers and

longer tubes around 650µm in more mature flowers. In thirty flowers with caps intact, there was no evidence of pollen tube growth.

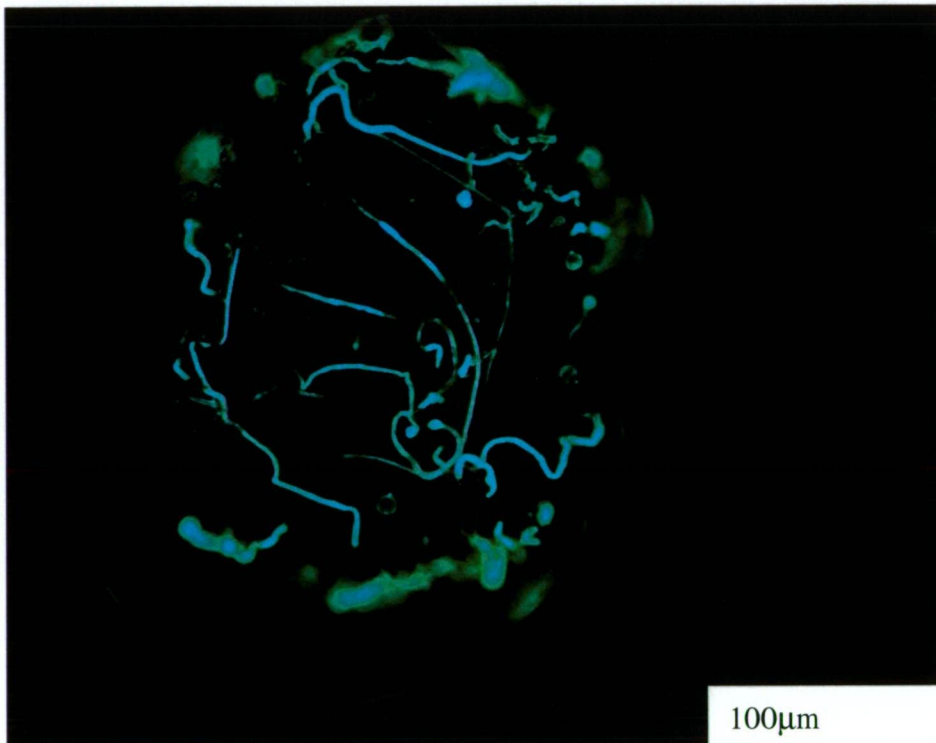


Plate 3.3: Fluorescent micrograph showing pollen tube growth on the stigma of an open flower

Self or Open Pollinated

There was no significant difference in fruit set between the self and open pollinated bunches. Fruitset of the self-pollinated bunches was 79.8% ($s = 6.7$) and in the open pollinated bunches 73.2% ($s = 7.3$).

The sequence of anther dehiscence is illustrated in the plates 3.4-3.7.



Plate 3.4: The beginning of cap fall, with the calyptra beginning to shed.

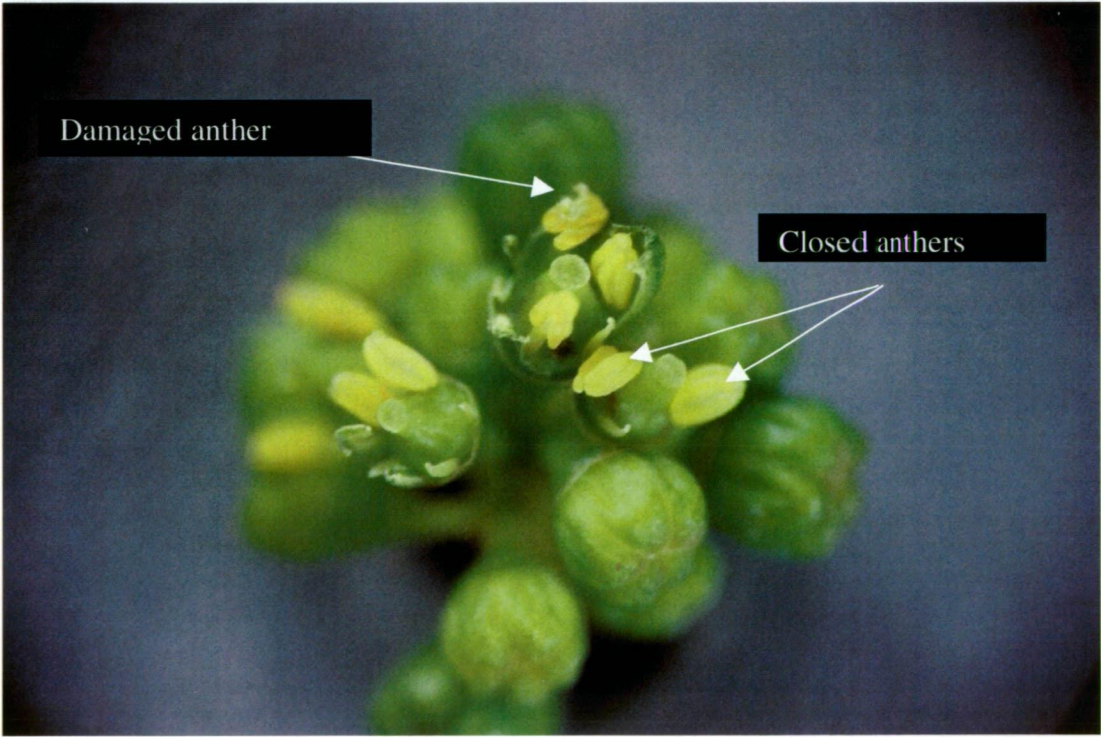


Plate 3.5: Flowers with closed anthers (pre anthesis). The caps of these flowers were removed manually.

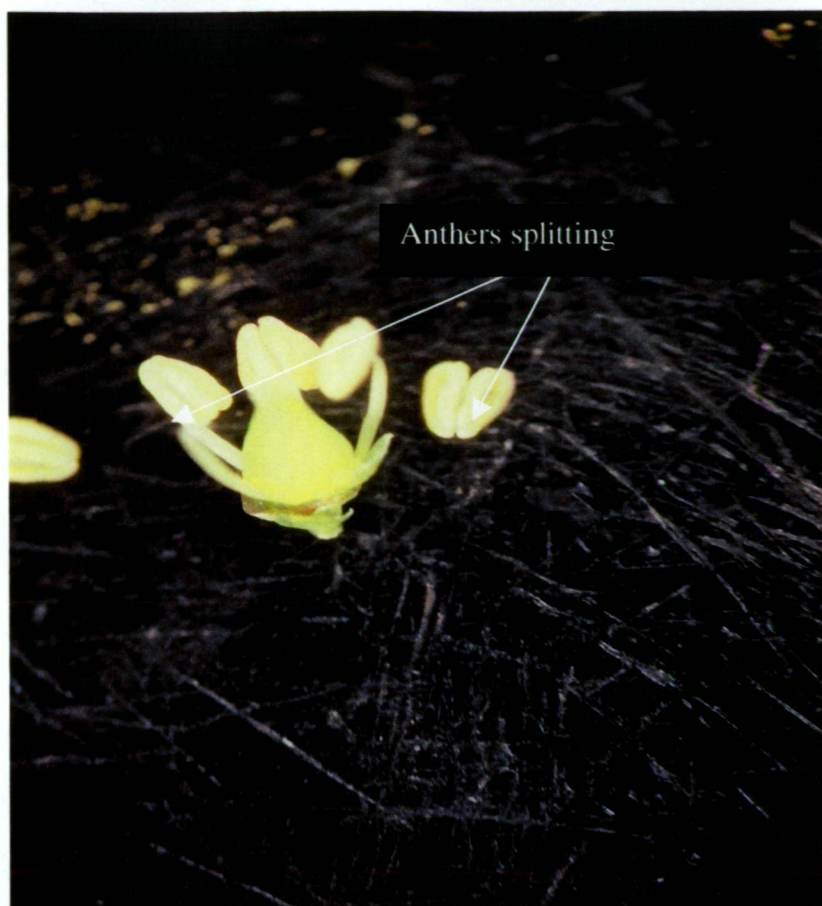


Plate 3.6 – A flower at anthesis, with anthers opening .

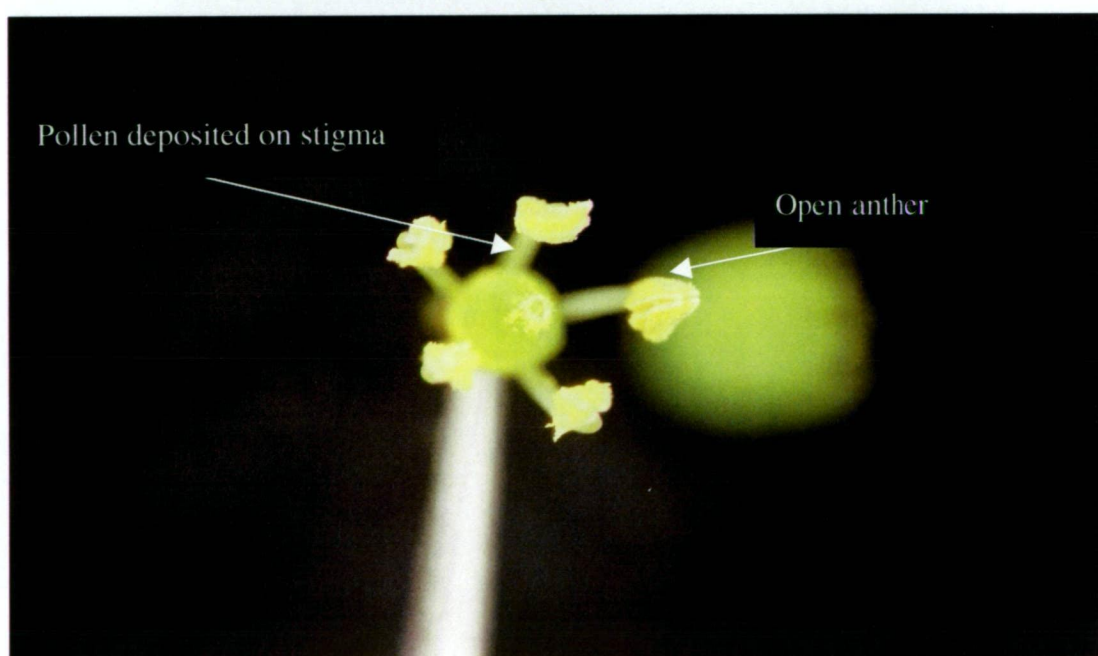


Plate 3.7 – After anthesis, anthers have opened completely and pollen has been deposited on the stigma

Fungicide toxicity

In vitro experiment

For each fungicide, the recommended commercial mixing rate diluted to 10% prevented any pollen germination. At lower concentrations, there was a significant interaction between fungicide and dilution rate for both pollen germination and tube length (Tables 3.2 and 3.3).

Table 3.2: In vitro germination percentage of pollen grains exposed to dilutions of fungicides. Figures with the same superscript were not significantly different based on the LSD (P=0.05) calculated for arcsine square root transformed data. Results with zero germination were not included in the statistical analysis.

Fungicide	Percent Germination			
	<i>Recommended Concentration</i>	<i>10% dilution of recommended</i>	<i>1% dilution of recommended</i>	<i>0.1% dilution of recommended</i>
Pyrimethanil	0	0	53 ^b	62 ^b
Cyprodinil + fludioxonil	0	0	25 ^a	48 ^b
Chlorothalonil	0	0	7 ^a	52 ^b
Control	62 ^b			

Table 3.3: In vitro pollen tube length for pollen germinated at the dilutions shown. Figures with the same superscript were not significantly different based on the LSD ($P=0.05$) calculated for untransformed data. Figures with zero growth were not included in the statistical analysis.

Fungicide	Pollen tube length (μm)			
	<i>Recommended Concentration</i>	<i>10% dilution of recommended</i>	<i>1% dilution of recommended</i>	<i>0.1% dilution of recommended</i>
Pyrimethanil	0	0	93 ^a	523 ^b
Cyprodinil + fludioxonil	0	0	80 ^a	366 ^b
Chlorothalonil	0	0	60 ^a	750 ^c
Control	800 ^c			

Dilution to 1 % allowed some pollen germination in each treatment with pyrimethanil allowing significantly ($P<0.05$) greater percent germination than either cyprodinil + fludioxonil or chlorothalonil (table 3.2). There was no difference in tube length between the three fungicides at the higher concentration (table 3.3). Tube morphology also changed, with tubes having swollen ends at higher concentrations (plate 3.8).

At the lowest concentrations germination and pollen tube growth were significantly greater compared with the higher concentration for all three fungicides. At the lowest concentration there was no significant ($P>0.05$) difference between fungicide effects on germination, and fungicide treatments were not significantly different compared

with the control. Pollen tube length was however significantly ($P < 0.05$) reduced by both pyrimethanil and cyprodinil + fludioxonil compared with chlorothalonil and the control.

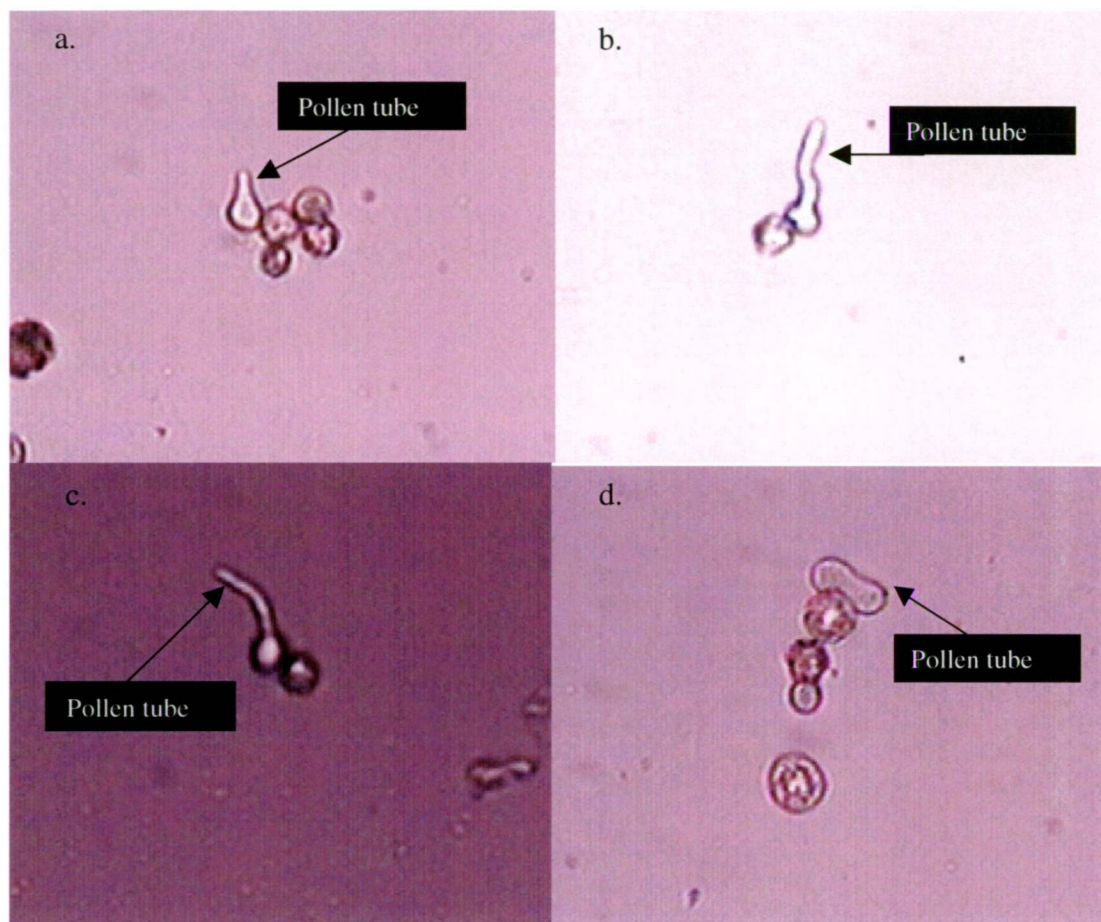


Plate 3.8 a-d showing pollen tube growth under different conditions.

- a. The beginning of pollen tube growth in nutrient solution.**
- b. Pollen tube growth in nutrient solution**
- c. Pollen tube growth in 0.1% fungicide dilution**
- d. Malformed pollen tube in 1.0% fungicide dilution**

Field experiment

Percent fruitset was not affected by pyrimethanil fungicide application at either stage of flowering and in all sample bunches reached around 90%. Average seed number per berry at harvest was however, affected by fungicide application with the average for the control 2.1, and the 5% cap fall treatment having 1.8 and the 80% cap fall treatment 2.2 seeds ($P=0.05$, $LSD=0.29$). All seeds in the control and 80% cap fall treatment were viable, while two berries in the 5% cap fall treatment had non-viable seeds.

The weather during the flowering season of 2002/03 was generally representative of previous seasons. The long term averages from 1952 to 2003 showed an average maximum temperature of 18.4°C for November, 20.1°C for December and 22.3°C for January, with average minimum temperatures of 7.0°C, 8.6°C and 9.4°C respectively and relative humidity 68%, 67% and 66%. Rainfall was however substantially lower than the long-term averages of 68.7mm, 63.3mm and 47mm for the three months.

Discussion

Method of pollination

Flowers set fruit when exposure to external pollen sources was prevented by bagging inflorescences from before cap fall, and there was no significant difference in set between covered and uncovered flowers. This result confirms that Pinot Noir flowers are self pollinated and self fertile.

Earlier findings or speculation that pollen dispersal is by wind or insects (Winkler, 1974) may be correct but as there was no difference in fruitset between bunches formed from inflorescences exposed to external pollen sources, the result appears to indicate that, for this variety, cross pollination is not necessary under normal conditions in Tasmania. Although Kimura *et al* (1998) reported an increase in fertilisation and consequent berry set with cross pollination in *Vitis coignetaie*, there appears to be a general acceptance that *V. vinifera* is self fertile and the present results support this view.

The presence of pollen on stigmas before cap fall indicated pollen dehiscence prior to flower opening, and that pollen germination and pollen tube growth only commence once cap fall has occurred. This conclusion was supported by the observed presence of open anther lobes in inflorescences prior to cap fall. The presence of open anthers in flowers with caps in place suggested that accidental damage to the anther and associated scatter of pollen during cap removal was not responsible for the

appearance of pollen on the stigma before cap fall. Dislodging of pollen from open anthers during cap removal could not be ruled out, but care was taken to minimise movement of flower parts during cap removal. Further, in all flowers examined as the calyptra had begun to shed (shown in plate 3.4) anthers had dehisced and pollen was present on the stigma.

Pollen viability tests showed that dehisced pollen was already viable underneath the cap but contrary to the results of Staudt (1999), there was no evidence of germination and pollen tube growth until after cap fall. By extrapolating from measured pollen tube growth Staudt (1999) calculated that 1 hour prior to cap fall, 35% of the flowers had pollen tubes, but this percentage decreased to less than 5% at 24 hours prior to cap fall. While unopened flowers may not have been sampled in the short timeframe before cap fall (1-24 hours) in the present study none of the 30 flowers sampled with caps in place showed any pollen tube growth. Observations of pollen tube growth were not made in the previous study, but rather the results were based on extrapolation using previously identified pollen tube growth rates of *Vitis rupestris* (Staudt, 1982). This species may have had pollen biology different to Pinot Noir or alternatively, the method of extrapolation based on the early stages of a sigmoidal growth curve may have indicated an earlier pollen germination than would normally occur. Thus, although Pinot Noir is self fertile, and pollen transfer occurs in the protected environment inside the capped flower, the evidence presented in this study suggests that pollen germination and subsequent pollen tube growth are delayed until after cap fall.

There were marked changes in the appearance of pollen grains and the stigma surface after cap fall. Before cap fall, elongated pollen grains were present and the stigma surface presented as apparently turgid cells. After cap fall the surface cells appeared more flaccid and the pollen grains had become more spherical in shape. These observations all agree with Miaja *et al* (1998-1999) who, working with the self-fertile cultivar Barbera, showed that fresh flowers before anthesis had turgid cells and there was pollen on the stigma before cap fall. Collapse of cells on fresh material was observed when the calyptra began to rise and pollen was already visible on the stigma. The authors also reported that, when the pollen tube elongated and the contents of the grain migrated through the pore, the pollen grain partially collapsed, leading to a more spherical appearance. Thus, although pollen tubes were not visible on the ESEM images obtained in the present study, the observed changes in pollen shape were consistent with observations reported by Miaja *et al*, (1998-1999).

The conclusion reached by Staudt (1999) that pollen tube growth commences before cap fall, implied that cap fall has no significant role in pollination and subsequent fertilisation. In contrast, failure of pollen to germinate under the cap, as shown in the present study, suggests that although flowers are at least partly self fertile and pollen transfer occurs under the cap, cap fall is an essential step in the processes leading to fertilisation. If this is the case, then research on factors influencing cap fall may give a useful guide to environmental influences on fruit set in this variety.

Fungicide toxicity

Pollen germination and subsequent tube growth were greatly affected by the presence of fungicides when germination was carried out on a nutrient solution *in vitro*. These results were consistent with previous findings for varieties Bolgar and Cabernet Sauvignon reported by Nikolov *et al*, (1999). The strong inhibitory effect *in vitro* did not, however, result in a significant reduction in fruitset in the field trial. There was however a small but significant reduction in seed number when this fungicide was applied at 5% cap fall but not at 80% cap fall.

In commercial vineyards in Tasmania, Pinot Noir has an extended flowering of 3-4 weeks. This combined with the link between cap fall and pollen germination after cap fall, established earlier in this chapter would result in a relatively small proportion of the total pollen yield in each bunch being directly exposed to fungicide at the time of application.

Systemic fungicides are transported by translocation in the xylem and phloem, with a very small amount of the compound reported to enter the anthers (Pavlik and Jandurova, (2000). Therefore, pollen grains exposed *in vitro* should be much more sensitive to potentially toxic compounds than under field conditions. After pollen shed, fungicides would have to make direct contact with the pollen grain or stigmatic surface to cause pollen toxicity. Kopcke *et al* (2002) also suggest that non-systemic contact fungicides would be unlikely to have an effect if the pollen tubes have already penetrated into the style.

Pyrimethanil and cyprodinil + fludioxonil are both systemic fungicides, so that in field grown plants, although direct exposure might be limited, ongoing exposure to systemic material in the anther or surface residue on the style would be expected, especially for application early in flowering at 5% cap fall. Chlorothalonil is a contact fungicide with multi-site activity, and being non systemic would be expected to have a lower residual concentration. Chlorothalonil inhibited both germination and pollen tube growth at higher concentrations, but unlike the other two fungicides had no significant effect on either germination or tube growth at the lower rate tested *in vitro*. This chemical, registered for use during flowering, may represent the lowest (of the materials tested) risk if in-vineyard pollen toxicity is an issue under specific conditions.

Church and Williams (1978) reported similar pollen toxicity on apple pollen and suggested that some chemicals can be less toxic to pollen lodged on stigmas than *in vitro*, due to dilution of the toxic material by stigmatic fluid. In either case it would appear that dilution of the fungicide either internally or on the surface of the stigma, may reduce the concentration to which the pollen is exposed below damaging levels resulting in little commercial concern and ongoing use of these materials.

In spite of minimal likely exposure of pollen under field conditions, there was evidence of a fungicide effect as seed number was lower at 5% cap fall, in the field trial. Whilst this did not result in reduced set in this trial, it does raise the possibility of reduced set under environmental conditions less favourable to good fruitset.

Yi *et al* (2003) found that fungicide sprays caused direct detrimental effects on stigma morphology and enhanced exudate production in almond flowers. Percent fruitset was not measured, however increased exudate production was raised as possibly causing inhibition or even preventing pollen tube growth and germination. The authors also suggested that the increased exudate production may have been a stress response, which could decrease the period of stigma receptivity. Cyprodinil promoted an increase in exudate secretion and caused the most severe collapse of stigmatic cells of all the fungicides evaluated in the almond study (Yi *et al*, 2003). This is an isolated report of fungicide effects on the stigma. As *Prunus* species commonly produce copious amounts of exudates in response to stresses (Bradley *et al.*, 1969; Morrison and Polito, 1985) the effect may be limited to this genus.

The susceptibility to fungicide damage of pollen in capped flowers needs further investigation. Pollen will be protected from direct exposure but movement of fungicides either systemically or by rain splash, or dew, after cap fall may result in exposure in flowers that were capped at the time of spraying.

Pollen tubes growing under fungicide concentrations, which reduced but did not completely inhibit pollen germination *in vitro*, showed markedly shorter tubes, and altered tube morphology. Exposed to fungicide diluted to 1% of the recommended spray concentration, many pollen tubes exhibited swollen ends. This was consistent with symptoms in *Tradescantia virginiana* L. (Spiderwort) pollen, where exposure to the fungicide benomyl caused tube rupture and swollen ends (He *et al.*, 1996).

Although field studies showed no significant reduction in fruitset, the *in vitro* tests demonstrate potential for pollen toxicity to occur. It appeared that prolonged flowering resulting in minimal direct exposure to fungicide prevented any significant effect of normal fungicide application on fruit set and consequently on final yield. The significant reduction in seed numbers for the 5% cap fall treatment may be explained by a greater number of pollinating flowers being open at the time of treatment, or exposed to fungicide residue following cap fall. That is, with fungicide application at 80% cap fall, most flowers will have completed fertilisation before exposure and only the remaining 20% can be exposed to residual fungicide.

Earlier results show that although anthers dehisced in closed flowers, pollen grains already on the stigma did not begin to germinate until the cap was ejected. Consequently recently opened flowers would be the most susceptible to damage caused by fungicide contact, and flowers that have been open for some time would not be susceptible to pollen damage.

It would be expected that in situations where flowering is reduced to a short period, or where cap fall is concentrated into a relatively short period within overall flowering, vulnerability of pollen to poorly timed fungicide application may be increased. Cool moist conditions which favour fungal growth and therefore require greater fungicide use may also delay or even prevent cap fall (May, 2004).

Consequently although the present results indicate little cause for concern, concentration of cap fall in an otherwise long flowering period may result in an unusually high direct exposure of pollen to Botrytis fungicides depending on time of

application. A further study into the possible damage to fruitset in grapevines by fungicide applications during flowering appears warranted, especially under cool damp, high disease pressure conditions. Prudent management would also suggest that application early in flowering should be avoided if it does not increase the risk of Botrytis infection.

Chapter 4 – Pre-flowering management influences on yield and fruit quality - canopy manipulation

Introduction

Fruit yield is made up of different components including the number of vines per hectare, number of canes per vine, number of shoots per bud, number of bunches per cane, number of berries per bunch, and individual berry weight.

Aside from the number of vines per hectare and the number of canes per vine, which are determined at planting and pruning respectively, all other yield components may be influenced by management practices as well as weather, at different times of the grapevine growth cycle. The number of bunches per shoot and flowers per bunch are linked to inflorescence initiation and flower development during the reproductive growth cycle. After flowering, berry number and size are controlled by pollination, fertilisation, berry retention and vine management during the current and previous seasons.

There has been an extensive range of studies on flower initiation, flowering, fruit set and berry development often containing inferences on weather effects. However, as noted earlier, major production areas do not experience the variable climate of Tasmania and researchers generally down-play weather effects in more stable climates (Winkler *et al.*, 1974). There are very few papers reporting the sum of such influences on yield of cane pruned vines in cool maritime climates. Consequently this chapter examines the effects of canopy management over three seasons on vine

fruitfulness. This literature review concentrates on general vine physiology and management principles influencing yield and does not attempt to canvass the range of information specific to spur pruned vines in warmer or more continental climates. The components of yield are discussed in regard to their impact on final fruit yield, as well as how training and trellising, carbohydrate partitioning and the balance between the reproductive and vegetative growth stages affect each of the components.

In view of industry concerns about an inverse relationship between yield and wine quality (Farquhar, 2003), fruit quality parameters considered important by local winemakers were measured on fruit harvested from the first pruning trial. While a range of external factors has been shown to influence wine quality, the interaction between management of pruning and canopy structure and the dominant environmental factors of temperature and sunlight appear to be the main controlling influence on quality. There is a wealth of literature on the interaction between canopy structure and sunlight interception and penetration for vines (Mabrouk and Sinoquet, 1998) and other species. The present review is not intended to cover options in planting design but aims to establish the likely links between development of major flavour components and canopy changes within the basic vertical shoot positioned structure. To determine whether the different pruning regimes had an effect on wine quality, anthocyanin concentration, sugar levels and pH were determined on fruit samples taken from the second vintage (2003) of Trial 1. The canopy management options used in the current study were tested within the general cane pruned vertical shoot position framework preferred by local growers. It

was beyond the scope and time frame of this industry funded study to consider major structural changes to commercial canopy management.

Background

Dry (2000) separated vineyard design influences on yield from physiological effects and listed the components of yield per node as shown in Table 4.1. The term node was used rather than yield / bud, which is commonly used by growers and other literature, but leaves some doubts about the status of nodes where a bud is missing due to damage or failure to develop. Yield / bud is used for the remainder of this thesis, but is equivalent to Dry’s use of node and should be interpreted as bud position.

Table 4.1 Components of yield (fruit weight)/bud

Fruit Weight / bud (1)	= Shoots/bud (2) x Bunches/Shoot (3) x Weight/Bunch (4)
Weight / Bunch (4)	= Berries/Bunch (5) x Weight/Berry (6)
Berries/Bunch (5)	= Flowers/Bunch (7) x Berries/Flower (8)

Of the controlling factors shown in the right hand side of table 4.1, all except (6) and (8) are potentially determined during compound bud development at the node (Dry, 2000).

The initiation and differentiation phases were reviewed in detail in Chapter 2. Briefly, from May (2004), anlagen (inflorescence initials) are formed in the bud

situated in the axil of every leaf of the young green shoot. At the earliest stage of development primordia of inflorescences are indistinguishable from those of tendrils. They are initiated 15 months or more before the resultant fruit is harvested (in Tasmania this is November / December two years before vintage). Initiation follows progressively in the buds along the green shoot. Anlagen develop into three types of primordia; inflorescence primordia, transition forms between inflorescence and tendril. Before the onset of dormancy, inflorescence primordia have branched to form an inner and an outer arm, initials of side branches. The transition from inflorescence primordium to inflorescence development commences in early spring the following year when the over wintering buds begin to swell. The phases of inflorescence initiation and differentiation can be influenced by internal regulation within the vine, through processes such as carbohydrate partitioning and hormonal regulation. However, the roles of the various endogenous growth substances in initiation and flower development remain poorly understood (May, 2004).

Carbohydrates and the growth cycle

Like most perennial species the woody tissue in grapevines is a storage site for reserves, and provides both carbohydrates and mineral nutrients for growth during the early part of the season following bud burst (Scholefield *et al.*, 1978). Once a sufficient leaf surface has been established by the emerging shoots, current photosynthesis becomes the primary source of carbon for growth and for replenishment of reserves. When sucrose begins to accumulate, that is, when the

rate of synthesis exceeds the rate of removal by the transport system, photosynthate is diverted into starch (Mullins, 1992) for both short and long term storage as starch grains in parenchymatous cells in the leaves, stems, and roots (Salisbury and Ross, 1985).

Starch is converted to soluble sugars to support base level metabolism over winter and (particularly) initial spring growth resulting in a spring minimum of stored assimilates (Mullins *et al.*, 1992). Early root growth, the development of spring buds, new leaf expansion and the early stages of flowering exhausts most of the stored carbohydrates. This low level of starch reserves is of relatively short duration as a fresh supply of photosynthate produced by the young leaves is allocated to storage beginning an accumulation towards the autumn maximum (Mullins *et al.*, 1992). Grapevine leaves reach about one third of their final size before they begin to export carbohydrates, but continue to import carbohydrates until half their final size with the direction of movement depending on sunlight levels (Mandel *et al.*, 2001).

The demands made upon resources in a perennial plant are greatest during periods of most active growth, and reserve materials are required in greatest measure when photosynthetic capacity of the vine is low, due to a small leaf area. Rapid growth is quite common at times when leaf area is relatively small (Mandel *et al.*, 2001).

Hence the number of buds relative to the accumulated carbohydrate storage at spring budburst will inevitably influence initial growth arising from the bud and for flower buds setting fruit it may therefore influence growth for the remainder of the season. As new growth is also linked to flower initiation, reserves at this time may also

influence bud development for the following flowering / crop development cycle (May, 2004).

The principle factors that influence accumulation of carbohydrate resources are the primary influences on photosynthesis; leaf area, light, temperature and water availability.

For a given carbohydrate reserve and current photosynthetic capacity, the availability of carbohydrate resources for individual buds will depend on the number of buds that are retained at pruning. If the number of buds exceeds the maximum which can be supported by the available carbohydrate supply, and adequate carbohydrate is assumed to be important for developing organs, it would be expected that one or a combination of outcomes would ensue; (1) fewer inflorescences would develop, (2) fewer flowers per inflorescence would form later in the season, or (3) some buds remain dormant at normal bud burst. Resource limitations would be compounded even further by competition between inflorescence development and vegetative growth of young shoots in a large canopy.

There is some evidence that not all grape cultivars depend equally on reserve materials. Zapata *et al* (2001) showed that Pinot Noir ceased using root stored starch earlier than Merlot, and had begun to renew root/stem starch reserves by the start of anthesis. This result suggested that, at fruitset, Pinot Noir would be affected less by low storage reserves. Zapata *et al* (2004) showed that in Pinot Noir roots, starch levels were lower than Merlot during dormancy, and although the level decreased in

Merlot at budbreak, it remained constant in Pinot Noir. After a rapid decrease in root starch levels in spring for both varieties, root starch levels began to increase again at the onset of anthesis in Pinot Noir but not in Merlot.

Yang *et al* (1980), from a study using ^{14}C as a tracer, suggested that in *Vitis vinifera* cv. Delaware, the translocation of the current year assimilates from the shoots to the lower parts of the vine began at about the 10-leaf stage and increased rapidly from flowering. However, it was not until the middle of July (seasonally equivalent to January in Tasmania), when xylem tissue began to be lignified, that the accumulation became pronounced. Carbon also accumulated in roots in a higher concentration than in the cane or trunk.

Throughout the growing season, there are fluctuations in the rate of accumulation of stored carbohydrates. Sommer and Clingeleffer (1995) noted a clear seasonal trend in the concentration of carbohydrate in wood tissue. The concentration was highest in mid-winter, lowest in mid-season and increased again towards the end of the growth period. Fluctuations may be due to different demands placed on the plant during different growth stages. These stages can be divided into vegetative growth including shoot and root extension and radial growth, leaf growth and reproductive growth including flowering and fruiting (Priestley, 1962).

In all seasonal analyses of carbohydrate resources in trees and vines, a drop in the carbohydrate content of the older regions of the vegetative structure has been recorded with the start of new season growth. Priestley (1962) stated that it is usual

for the first leaves to unfurl from winter buds before shoot extension begins. This is then followed by a rapid phase of shoot elongation. This early vegetative growth phase would correlate closely with the carbohydrate pattern with the new leaves beginning to photosynthesise soon after their appearance. Thus, part of the initial drain on reserves during shoot growth contributes to the formation of new leaves, which in due course enable the new shoot to become self-supporting.

In common with most temperate perennial crop species, flower initiation in grapevines takes place a full season before flower emergence. Flower initiation occurs during early summer (Coombe, 1989) when shoot elongation has almost ceased and reserve carbohydrates have begun to replenish. A period of differentiation follows and is thought to halt during winter dormancy and resume again at bud burst. Therefore the final stages of flower differentiation utilise stored carbohydrates in competition with bud burst, early leaf growth and the commencement of shoot and root elongation. Young vegetative organs generally represent stronger sinks than reproductive structures in grapevines, suggesting that the latter stages of floral differentiation would be competing directly with the stronger sink of shoot elongation (Fournioux, 1997); cited in (Zapata *et al.*, 2004). Flower clusters are also reported to compete for carbohydrates with the shoot apex during cap fall and after anthesis when the developing clusters become strong sinks (Smithyman *et al.*, 1998).

After fruitset and during berry development, growing fruits use carbohydrates from both current synthesis and storage, depending on the variety (May, 2004). The demand placed on stored and current carbohydrate can have an effect on both the

crop in the following year as well as the vegetative growth extending into the following season. Priestley (1962) stated that, in fruit trees such as apples, heavy cropping in one year can lead to a small fruit crop in the subsequent year, which may be due to the absence of sufficient reserves to support flower initiation. Termed irregular or biennial bearing, this alternating heavy / light crop cycle has not been reported for *Vitis vinifera* but as noted earlier biennial bearing has recently been reported in *Vitis labrusca* by Watson (2000).

Carbohydrates and vineyard management

In vines, premature leaf fall can occur as a result of water stress in autumn with unfavourable seasonal conditions, pest or disease attack or mechanical harvesting. Cultural practices such as leaf plucking, during active growth may also have an impact on carbon assimilation. The premature arrest of leaf function is likely to prevent both post-harvest acquisition of reserves as well as nutrient resorption from the leaves that occurs during normal leaf senescence. For example, May (2004) quoted results showing that Semillon vines deprived of leaves at harvest, had reduced shoot growth and a lower crop, as less berries per bunch, in the following season.

As well as premature leaf fall, increased vegetative growth due to fertiliser application can also influence reserve carbohydrate accumulation. Application of nitrogen fertiliser usually promotes vegetative growth, causing a short-term reduction in reserves as a result of incorporation of nitrogen into amino acids and protein (Priestley, 1962) with enhanced vegetative growth. Whilst nitrogen has the most

widely reported effect on carbon accumulation and allocation, all of the nutrient elements associated with carbohydrate metabolism and transport may have a similar influence.

Pruning can have a direct effect on carbohydrate concentration. Winkler (1929) showed that lighter pruning of grapevines resulted in an increased concentration of carbohydrates, measured as the soluble sugar fraction, during the following growing season. Less new vegetative growth was removed by light pruning, but the increase in carbohydrate was attributed to fewer active meristems competing for reserves in more heavily pruned vines.

Sommer *et al.*, (1995) reported that vines receiving minimal or no pruning were often found to have accelerated canopy fill compared with traditionally pruned vines.

Winkler (1958) had previously argued that such enhanced development was a result of improved carbohydrate storage with minimal pruning, compared with that of traditional cane or spur pruning of grapevines, which removes a large proportion of the wood production. Depending on the pruning severity, up to 95 percent of wood and a proportional amount of carbohydrates, mainly in the form of starch and sugars are removed (Winkler, 1958; Winkler *et al.*, 1974). The proportion of total carbohydrate removed is not, however, directly proportional to the proportion of canes removed at pruning, with the trunk and root systems accounting for a (usually) undetermined storage capacity.

A more recent study conducted by Sommer and Clingeleffer (1995) in a warmer region than Tasmania, found that neither the concentration nor composition of carbohydrates was strongly influenced by minimal or traditional pruning systems. However due to the larger canopies formed under the minimal pruning system the total mass of carbohydrates per vine was greater than the traditionally pruned vines.

As would be expected the literature confirms that management processes, which adversely affect the synthesis of carbohydrate either directly or indirectly, can have a negative impact on carbohydrate accumulation and storage. Direct influences can be due to processes such as leaf plucking, which not only interrupt synthesis but also prohibit nutrient resorption from senescing foliage (May, 2004). However, other research reviewed by Caspari *et al.*, (1988) has shown that loss of leaf area due to leaf removal may be compensated for by increased photosynthetic efficiency in the remaining leaves (Hofacker, 1978; Hunter and Visser, 1988a); cited in (Caspari *et al.*, 1998), an increase in lateral leaf area (Kliwer and Fuller, 1973; Hunter and Visser, 1988b); cited in Caspari *et al.*, 1998), and a delay in leaf senescence and abscission (Candolfi-Vasconcelos and Koblet 1990); cited in Caspari *et al.*, 1998). Indirect effects can be varied and may include nutrient applications and pruning severity, which will impact on canopy size and hence leaf area which in turn affects potential photosynthate assimilation. However, recent results show that pruning systems do not impact on carbohydrate concentration or composition, but instead it is the total mass of carbohydrates per vine, which is affected due to canopy size (Sommer and Clingeleffer, 1995).

Management of both vegetative growth and reproductive phases influences carbohydrate concentration and availability. Flower and fruit thinning have a direct influence on carbohydrate availability. In Burgundy for example, bunch thinning before veraison is sometimes practiced to improve the quality of the two main cultivars Pinot Noir and Chardonnay Blanc (Bessis *et al.*, 2000). Removing flowers or fruit increases the amount of reserve material available for allocation to remaining sinks. Bravdo *et al* (1984) showed an increase in pruning weight from vines with fruit load decreased by bunch thinning. Edson *et al.*, (1995) found that root dry weight at fruit harvest was inversely related to fruit load, and that sugar accumulation and shoot maturation were delayed by high fruit loads. Since roots are said to be poor competitors for photoassimilates, the root:shoot ratio is generally decreased by heavy fruit load (Rodriguez-Lovelle and Gaudillere, 2002) and the total available store of carbohydrate for the following season will in turn be affected.

Overall vineyard practices, which affect both vegetative and reproductive growth have the potential to alter the ability of the vine to synthesise, store and, in the following season, make available carbohydrates, thus influencing both yield and quality.

Hormones and internal regulation

Regulation of flowering in woody perennials remains poorly understood, and a review of the literature is beyond the scope of this study. However, a recent paper by

Boss *et al.*, (2003) gives a new perspective based on the fact that the floral stimulus is repressed in actively growing grapevine tissues. It is suggested that a major influence on grapevine flowering is an endogenous signal that inhibits the differentiation of uncommitted primordia into inflorescences on growing shoots (Boss *et al.*, 2003). Thus, flower initiation is primarily a response to removal of inhibition rather than any promotive influence. Previous research on various hormone groups has been generally inconclusive. The involvement of auxin in flowering and fruitset has been described as direct, or indirect through its relationship to ethylene metabolism, however May (2004) noted that with very few exceptions, research of auxin effects in flowering and fruitset in grapevines has not progressed since the work of Nitsch *et al.*, (1960). The lack of research on auxin concentrations during flower development, flowering and fruitset was highlighted.

Gibberellins and cytokinin synthesised in roots may be important in controlling shoot and flower growth (Niimi and Torikata, 1978). Decreased gibberellins or increased cytokinin levels may promote the development of inflorescences (Srinivasan and Mullins, 1981). A requirement for importing cytokinin into the flower to support the growth of the pistil was shown in tissue culture experiments by Pool (1975).

Gibberellin is involved in the regulation of many plant processes, including fruit development (May, 2004), and recent genetic evidence suggested that gibberellins are major inhibitors of floral meristem production in grapevines (Boss and Thomas, 2002). Using a mutant grapevine with altered floral induction genotype, all uncommitted primordia had greater potential to become inflorescences, regardless of

the environment in which they were formed and, in optimal conditions, inflorescences and bunches were formed along the length of the shoot.

The above literature suggests that hormones and internal regulation can have an affect on both the vegetative and reproductive cycles of the grapevine, which in turn may affect the fruitfulness and overall yield of the vine. In particular, the suppression of flowering by gibberellins suggests that the competition between vegetative and reproductive growth may be mediated by gibberellin as well as, or instead of, supply of reserve carbohydrate and current photosynthate.

Growth pattern effects on inflorescence initiation

The relationship between temperature and inflorescence initiation has been widely documented. Alleweldt (1963) (cited in Mullins *et al.*, 1992) showed that there was a negative relationship between temperature from mid to late summer and the number of inflorescences appearing on the shoot in the following season. Cabernet Sauvignon and Merlot vines, when exposed to 12°C until after budburst, produced significantly more flowers and, in Cabernet, longer inflorescences than when exposed to 25°C (Pouget, 1981) or 28°C (Ezzili, 1993); cited in May, 2004). Pouget assumed that the lower temperature regime favoured inflorescence growth by disadvantaging shoot growth. This was accentuated in Cabernet Sauvignon because its budburst phase is longer than that of Merlot. In contrast, Ezzili (1993) suggested that the number of flowers differed because some failed to develop fully at the higher temperature. May (2004) proposed an alternative explanation, suggesting a role for

cytokinins which are promoted at higher temperatures. He proposed that higher temperatures cause a cytokinin enhanced enlargement of the early produced flowers which, in turn, inhibit the formation of the other flowers.

Growth pattern has an effect on inflorescence initiation. Pruning to remove shoots and canes in winter has been the main practice used to regulate vine morphology and yield of the subsequent crop. Most vines are pruned to a specific canopy design, which becomes the permanent vine framework and this determines the ongoing pruning management system. The main objectives in setting up a pruning/training system are to maximise production, facilitate cultural operations in the vineyard, improve canopy microclimate, and support the load of fruit on the vine (Coombe and Dry, 2000). Much of the published research on canopy light microclimate has been related to wine quality (for example Smart, 1988), but several references report effects on yield as bunches per vine or berries per bunch.

The major emphasis of canopy management is usually to reduce excessive canopy shading in order to maximise fruit exposure to light and hence wine quality, while maintaining an adequate balance between the amount of vegetative and reproductive growth (Smart, 1985). Canopies with a structure that provide an even bunch spacing have been shown to be more productive, when a range of vine spacing and trellising treatments were compared, than widely spaced rows with narrow vine spacing (Hedberg and Raison, 1982). The annual average yield per vine increased with decreasing vine density. Closer vine spacing decreased bunch weight and berries per bunch, but inter-row spacing did not affect bunch weight (Hedberg and Raison,

1982). The authors commented that increased vine density imposed through narrow vine spacings may have lead to inter-vine shading, as well as increased competition for soil nutrients and moisture, which may in turn have influenced inflorescence initiation, flower differentiation and possibly fruitset and berry growth.

Basal bud fertility has also been demonstrated to be affected by training and trellising, in turn increasing bunch number per vine. Doubling the canopy length by adding a second cordon training wire in trellis experiments in New York and Australia, produced major yield increases, resulting from increased fruitfulness of basal buds. The effect was attributed to reductions in shoot crowding (Kasimatis *et al.*, 1980). The response was attributed to an improved light regime at the basal portions of shoots.

Pruning has an effect on inflorescence initiation and floral differentiation. If more buds were left on a vine at winter pruning, fewer formed shoots the following spring (Jackson *et al.*, 1984). Some of these vines however were still able to respond by increasing yields in direct proportion to the higher bud numbers, by having more bunches on each node. It was found that the major contribution to this fruit increase was bunch number, although individual bunch weight made a small contribution (Jackson *et al.*, 1984). This result is consistent with bunch number as the variable exerting primary control of yield.

The most common trellis designs used in Tasmania are vertical shoot positioned (VSP) systems, including the horizontally divided modified lyre, and vertically

divided Scott Henry systems. The VSP system is designed to train the shoots into a narrow vertical canopy, and by raising a foliage wire as the shoots grow in spring, new shoots are retained into a narrow vertical wall. In the lyre system the walls of foliage are slightly inclined with all shoots positioned upwards. The vertical division in the Scott Henry system has shoots trained vertically upwards on a higher fruiting wire as well as shoots trained vertically downwards on a lower fruiting wire.

Most vineyards have row spacings, ranging from 1.2 to 2.4m and vine spacings of 1 to 1.2m, whilst lyre trellised vineyards tend to have wider rows (3.6m). These basic trellis types have not been objectively compared under local conditions and there is little anecdotal evidence to indicate any differences between them. Generally management decisions between the systems are based around practical issues with Scott Henry systems able to be mechanically pruned. Some growers believe the modified lyre system leads to better fruit quality due to increased light interception, but this has not been formally reported and the divided canopy presents some serious management problems particularly with access and spray penetration.

Generally the trellising/training system is determined at planting with ongoing canopy management dependent on controlling shoot number by altering the pruning level (Dry, 2000).

Effects of pruning and training on wine quality

The major components of quality are flavour, aroma and pigment. Although there have been many research studies attempting to objectively predict the flavour and aroma of a wine from the fruit at harvest there has been limited success. In contrast the pigment of harvested fruit has been shown to correlate strongly with that of wine (Hunter and de La Harpe, 1987; Iland *et al.*, 2000).

Sugar and pH are two quality parameters that are readily measured in harvested grapes, with the former often measured routinely as an indicator of maturity in commercial vineyards. Sugar in the must (grape juice before fermentation) is directly related to the alcohol content in the wine, which is important for economic and for sensory value (Sinton *et al.*, 1978). As the alcohol concentration increases during fermentation it improves extraction of alcohol soluble flavour and colour components from grape juice and for wines fermented “on skin”, from the berry pulp (Winter, 2001).

Grape acidity is widely regarded as an important characteristic determining wine quality (Jackson and Lombard, 1993). Low pH in wine is said to lead to better colour and to prevent spoilage (Winter, 2001). The total acidity measured as titratable acidity indicates available concentration of organic acid ions in solution and is mainly the sum of tartrate and malate, and measures both free-acids and salt forms. The pH (that is the concentration of free hydrogen ions) is not necessarily directly proportional to titratable acidity and two wines with similar titratable acidity may have very different free hydrogen ion concentrations.

Phenolic compounds composed mainly of anthocyanins and tannins in grapes are considered the most significant contributors to wine quality (Jackson and Lombard, 1993). Of these compounds, anthocyanin pigments extracted from the skins of red grapes during wine making are responsible for colour, arguably the most obvious quality component in red wine. Enzymes naturally present in grapes, or added as processing aids, break down the cellular structure of the cuticle and release cell contents, including pigments into the wine. Ethanol produced as a fermentation product also assists in this extraction (Asenstorfer *et al.*, 1999).

That high yields lead to lower wine quality is a commonly held view in many wine producing regions (Smart, 1980). There are several yield-stimulating practices thought to have negative effects on wine quality including irrigation, invigorating rootstocks, fertilisation, minimal pruning and high soil fertility. Therefore, based on these assumptions, if a grower is aiming for high quality wine, pruning and training techniques need to be directed towards setting up low yields and maximising light interception in the whole canopy. Canopy shading has been reported to produce high pH and a much lower wine quality, and low density trellis systems have been shown to produce generally better quality (Smart, 1980). In contrast, other researchers have found no difference in berry pH content of light exposed and shaded bunches (Dokoozlian and Kliewer, 1996).

The major influences of pruning and trellising on wine quality are generally accepted as being associated with exposure to sunlight of leaves and bunches (Mabrouk and

Sinoquet, 1998). Biomass production and consequently yield potential, have been shown to be related to the total amount of solar radiation intercepted by the foliage (Mabrouk and Sinoquet, 1998). However distribution and quality of light within the canopy appears to have an effect on wine quality, which is independent of total light interception by the overall canopy.

Jeong *et al* (2004) showed that shading suppressed anthocyanin accumulation. These authors reported that shading suppressed or retarded activity of a regulatory gene for anthocyanin biosynthesis in grapes.

In addition to effects on wine quality in the current season, pruning can also influence flower initiation for the subsequent seasons production, affecting both yield and quality. There is the possibility that increased shading of shoots and developing buds, may reduce flower initiation, with a consequent reduction in next years potential crop.

Kliewer (1968) regarded temperature as the primary determinant of quality in wine grapes. The greatest effect is on sugar accumulation and generally high temperatures during the ripening period are associated with grapes high in sugar and low in total acidity (Kliewer and Lider, 1970). Jackson and Lombard (1993) suggested that poorer quality associated with warm climates is at least partially due to low total acidity and high pH. Kliewer and Lider (1970) reported that increased rates of respiration associated with high temperatures most probably accounted for the striking decrease in total acidity and high pH of juice from fruit grown in high

temperatures. It has been thought in the industry that warmer climates also produce less flavour and aroma constituents, but to date little scientific data has been provided to verify these assertions and, overall, wine scores are higher from grapes grown in cooler areas. Kleiwer and Lider (1970) showed that low temperature reduced malate, proline, arginine and total N in berry juice. Temperature has also been shown to effect colour development. With evidence suggesting that temperatures too cold or too warm are associated with poor colour (Iland and Fetzmann, 2000). However the same study showed that relatively low daytime temperatures greatly increased the level of anthocyanins in skins of Pinot Noir.

The effects of light interception on grape berry development and composition are complex (Crippen and Morrison, 1986) and, as discussed below, inextricably linked with canopy architecture. Overcast skies can limit light exposure sufficiently to reduce net photosynthesis and influence berry composition. Conversely, high light intensity and increased duration of sunlight has been shown to increase yield and or sugar content (Archer and Strauss, 1989).

Higher radiation may also increase temperature, especially of exposed leaves and berries, leading to increased photosynthesis and metabolic activity. Solar heating of grape berries may increase cellular respiration and water loss, and both heat and light may affect the accumulation of anthocyanins and other phenolic compounds. Kliewer and Lider (1970) suggested that low light intensity significantly reduced colouration of Pinot Noir grapes, that is, they have a lower concentration of anthocyanins in the skins. They also showed that low light intensity resulted in reduced total soluble

solids, pH and proline, and in increased levels of total acidity, malate, arginine and total N in the berry juices.

Other studies have shown contradictory results. Much of the contradictory evidence in the literature regarding the effects of sunlight on the composition of grape berries undoubtedly is due to seasonal variability as well as to differences among studies in location, soil type, canopy manipulation, and variety (Crippen and Morrison, 1986). It may also be due to a failure to adequately separate light and temperature effects, as exposure of bunches to high light intensity will generally correspond with higher berry temperatures.

Findings by Jackson and Lombard (1993) suggest that high pH, characteristic of poorer wine quality, is generally associated with internally shaded canopies. Smart (1985) considered that bunch exposure was the critical issue for the pH and K⁺ balance. In contrast, a study by Dokoozlian and Kliewer (1996) found no difference in berry pH or K⁺ content between light-exposed and light-excluded bunches. A shaded environment has also been shown to increase the pH and K⁺ content of the must and reduce both wine colour and content of phenolic compounds (Iland, 1988). Shabala and Wilson (2001) demonstrated a light mediated control of pH and potassium flux in mature berries confirming the metabolic links between pH and potassium postulated in earlier work.

Practically, shade, whether natural or artificial, nearly always reduces sugar levels and usually increases pH, a response which normally results in delayed maturity.

Therefore training systems have been developed to minimise shading by spreading out the canopy to permit greater light penetration and therefore reduce both berry and leaf shading. Smart (1980) showed that shaded Shiraz vines produced high pH musts and much lower wine quality, and that the low density “Geneva Double Curtain” trellis produced musts and wines of low pH and generally better quality. These results were discussed only in terms of light exposure and the author did not note any issues associated with increased humidity, decreased air circulation and potentially increased disease.

Smart (1985) reported that shade causes increased pH of must and wine from both red and white winegrapes, and in hot and cool climates. Of the common fruit composition measures, Smart (1985) reported that pH appears to be the most readily affected by the light microclimate. The importance growers attach to light microclimate is evident in the widespread use of leaf plucking to improve bunch exposure.

The effect of shading on pH and other factors of fruit quality remains an issue of contention, but a recent study by Shabala and Wilson (2001) has identified a light sensitive link between potassium and proton fluxes in excised berry tissue. On balance there appears to be little doubt that high yields do lead to reduced wine quality, but the mechanism and role of the canopy light microclimate remain poorly understood. There is no obvious reason why heavy crops should suffer poorer direct exposure to light and current vineyard practice may confuse two separate issues. A heavy yield may limit availability of photosynthates and secondary metabolites from

the leaves, thus reducing quality. In contrast a low yield carried in a dense canopy may be shaded and fail to develop flavour characteristics associated with exposure (of the fruit) to direct sunlight.

Crop management to control yield and quality

The current view in the local industry is that premium wines are usually made from low yields, of to 6 tonnes/ha or less. A medium fruit yield is thought to be 10 tonnes/ha and a heavy fruit yield 14 tonnes/ha (Farquhar, 2004). Thus, although small variations in canopy structure, albeit at added pruning cost, may enable yields to be managed to economically sustainable levels, this would have to be achieved without compromising quality.

(Miller *et al.*, 1993) reviewed the concept of vine balance. The theory of balanced pruning is based around the concept that the greater the vegetative growth of the vine, the greater the capacity for fruit production the following year (Partridge, 1926). According to Miller *et al* (1993) “balanced pruning is based on the premise that a larger vine can mature a larger quantity of fruit than a smaller vine, and still provide enough carbohydrates to the roots, trunk and shoots to allow adequate carbohydrates for vine growth in spring until the leaves are fully functional”. It was also suggested that a vine of a given weight has the potential to assimilate a fixed amount of carbohydrates during a growing season. If the bulk of this is partitioned to the fruit, the overall plant weight can decline and the fruiting capacity will be reduced the following year.

Smart (1995) suggested that the optimum balance between fruit and winter pruning fresh weights was about 5:1. Ratios below or above this value indicate a vine that is out of balance. The actual amount of leaf area necessary to mature fruit has been reported to be approximately 10cm²/gram of fruit (Smart and Robinson, 1991).

However, in a field situation, trellis type, row orientation and canopy development all influence intra-canopy shading and the proportion of leaves that are exposed for photosynthesis (Petrie *et al.*, 2000a). Notably, there appears to be little comment in the literature on local variations due to daylength, cloud cover, sun angle and evaporative demand.

Crop yield is widely accepted in industry as an important factor in the quality of resultant wines, but Chapman *et al* (2004) was of the opinion that most prior research has shown no effect of yield on wine quality. There is however some research to support the general view, for example: Lower fruit loads in several red wine varieties were shown to produce highly coloured fruits and higher soluble solids but pH was unaffected (Gao and Cahoon, 1999). However, in a pruning trial using Zinfandel, the strongest correlation between quality attributes and yield was with pH (Sinton *et al.*, 1978). Morinaga *et al.*, (2003) noted that high crop load caused berries to have lower sugar content. Some authors have reported yield modifies the relationship between yield and grape composition in two ways, either directly (for example in competition for resources) or indirectly by altering the rate of development. Since high yields delay maturity (Jackson, 1987), development of grapes in cooler production regions

may be slowed until late autumn where there is inadequate heat to complete the ripening process, and wine quality suffers accordingly.

Balasubrahmanyam *et al* (1979) showed that high yielding vines produced grapes with less anthocyanins, aroma, and flavour constituents. The author was able to further confirm this by demonstrating that bud thinning prior to veraison lead to improvements in wine quality. Jackson, (1987) suggested that high yielding vines, because they mature later, should be picked later. If this is done, and seasonal conditions allow, quality may not be decreased.

Bunch thinning to manipulate yield conducted on Cabernet Sauvignon vines showed that aroma and flavour responded to yield manipulation significantly only when yield was altered early in fruit development. When bunch thinning was carried out at veraison, in general negative veggie attributes decreased in intensity and beneficial fruity attributes increased in intensity as bud number and yield increased (Chapman *et al.*, 2004).

The level of phenolic compounds in grapes has been found to be affected by different pruning severities, with lower numbers of buds leading to superior colour expression (Hunter and de La Harpe, 1987).

In papers reporting a relationship between yield and wine quality, the issue of vigour and cropping capacity have not always been taken into account as noted by Bravdo *et al.*, (1985). Winkler *et al* (1974) (cited in Bravdo *et al*, 1985) demonstrated that fruit

quality and yields both increased concomitantly with vine cropping capacity due to regulation of pruning severity and fruit thinning. Decreasing yields from 26 tonnes/hectare to 12 tonnes/hectare by bunch thinning in an experiment conducted by Bravdo *et al* (1985) were associated with increased berry weight, but the number of berries per bunch did not increase, and wine quality was not improved.

Winegrape quality is thought to be improved by growing grapevines under somewhat limited nutritional conditions (Rodriguez-Lovelle and Gaudillere, 2002). Such conditions slow down summer vegetative growth in order to favour berry maturation (Matthews *et al.*, (1987) and McCarthy, (1997). Again there is no clear distinction, in the literature, between stage of maturity and direct effects on accumulation of quality components.

Bravdo (1984) suggested that it may well be that inconsistent reports of the relationship between yield and quality may have occurred because fruit yield alone is an insufficient measure of crop level. Such a measure should be related to cropping capacity, a term which represents both vegetative growth and crop yield.

Crop, or fruit load and its effects on wine quality, canopy development and vine capacity has been widely documented. As a general rule, fruit load can be defined as the ratio of reproductive to vegetative tissues during the same growing season (for example Miller, 1993). Using this definition, a high fruit load is one with a high number of bunches per shoot, and a low fruit load with few bunches. "Shoot" in this definition is equivalent to the number of canes in the canopy in the current season.

High fruit loads may delay wood maturity, reduce the level of carbohydrates in the roots and adversely affect winter cold hardiness (Edson *et al.*, 1995). High fruit loads have been shown to significantly reduce the leaf area available for producing photosynthates in current or succeeding seasons. Put simply, with a heavy fruit load fruit is produced at the expense of vegetative structures in the current season. This relationship can be extended to the following year, as a vine of a given leaf area has the potential to assimilate a fixed amount of carbohydrates during a growing season. If the bulk of this is directed to the fruit, the overall plant weight can decline and the fruiting capacity will be reduced the following year (Miller, 1993). Recently, high fruit loads have been shown to reduce later shoot growth and reduce production of new fine roots (Morinaga *et al.*, 2003).

High fruit loads can result in shorter inflorescences and a smaller leaf area per inflorescence (Miller and Howell, 1998). Flower number per inflorescence has been shown to positively correlate with inflorescence length so that berry number per bunch is reduced in short bunches due to fewer flowers (Miller and Howell, 1998). In addition, a reduction in the percentage of flowers setting fruit may occur as a result of a reduction in leaf area per bunch at anthesis, which reduces carbohydrates (Miller and Howell, 1998) available for fruitset.

Miller (1993) showed that bunches per bud, berries per bunch and berry weight decline as bud number increases, and concluded that the effects were due to increased competition for available carbohydrates. Miller and Howell (1998)

repeated this finding, showing that high fruit loads lead to smaller bunch size and fewer berries per bunch.

Weather at particular stages of growth may have a significant impact on vine balance. Temperature, sunlight and rainfall have been shown to influence initiation of inflorescences, which sets the upper limit for yield (Baldwin, 1964; Buttrose, 1969b; Buttrose, 1974; Palma and Jackson, 1981). These same weather variables will also have an impact on carbohydrate accumulation both directly through the regulation of production and mobilisation of sugars, and indirectly through the drain of carbohydrates by large fruit loads.

The results of chapter 2 illustrated that weather at the time of flowering in some vineyards strongly influenced fruit yield, but that there was no weather effect evident during the period of inflorescence initiation. This suggests an influence on bunch size, rather than on the previously identified primary determinant of yield, bunch number. Further, management effects overriding seasonal patterns may be due to the commonly employed practise of bunch thinning in most vineyards.

Analysis procedures for anthocyanin pigment

Anthocyanins are associated with colour of berry skins in red and rose cultivars of grapes (Revilla *et al.*, 1998). Different methods have been proposed for extraction, but the method can play a major role in the extraction efficiency and the resulting estimate of concentration. Revilla *et al.* (1998), in their review of extraction procedures, reported that at low pH, anthocyanins are predominantly present in the

form of a flavylium cation, giving a predominantly red colour in aqueous solutions, thus easily visible during extraction processes. The flavylium cation is red and stable in a highly acid medium, and this chemical feature of anthocyanins has probably led to routine use of solvents containing mineral or organic acids for the extraction of anthocyanins from plant tissues. They also reported concerns about partial or total hydrolysis of some acylated anthocyanins, as well as generation of anthocyanidins from flavanols which both result in misrepresentation of total anthocyanin content. However Mazza *et al* (1999) showed that Pinot Noir grapes contain only nonacylated anthocyanins therefore eliminating the risk of using acidified solvents. This was also reported by Arozarena *et al* (2002).

As anthocyanin accumulation occurs in the skin of red grape (Pirie and Mullins, 1977), this presents a sampling problem for pigment determination. Some published procedures have included whole grape samples. However a study concerning treatment affects on anthocyanin accumulation should arguably only use grape skins, thus eliminating the compounding effect of varying pulp to skin ratio.

Experimental approach

The yield management strategy of fruit thinning is widely practised in other tree fruit crops (Davis *et al.*, 2004), where potential fruit is removed to a desired yield target midway through the fruiting cycle, usually at around colour development. Routine crop regulation is not commonly reported in the viticulture literature, but is

occasionally used where seasonal conditions result in higher than anticipated crops.

Anecdotal evidence suggests that it is more widely practiced in commercial vineyards in Tasmania than in other viticultural regions.

If growers were able to winter prune to an “above target” yield; then if necessary reduce the yield to near target levels based on bunch number and projected bunch weight once fruitset was complete, it might be possible to achieve target yields in most seasons. This practice would only be commercially acceptable if it is easily manageable and does not adversely affect wine quality.

The following two trials were designed to examine the effects of pruning to four prescribed bud numbers per vine on the yield components bunch number, bunch weight, berry number and berry weight. In addition, in a second trial, bunches of fruit were removed at veraison to test the effect of bunch thinning on the same yield components and to gauge the potential accuracy of thinning to a target yield.

For this pruning and thinning strategy to be effective, the influence of over-cropping on bud fruitfulness (as bunch number and size) in both the current and following seasons needs to be established. The concentration of carbohydrates in grapevines was first reported by Muller-Thurgau (cited in (Schaller, 2000) as one physiological factor that may influence fruitset and subsequently yield. Commenting on the relationship between storage carbohydrates and fruit yields, Edson (1995) reported that high fruit loads are associated with reduced levels of storage carbohydrates. In view of uncertainties about biennial bearing as a factor in yield variability, the effect

of early season over-cropping on yield components the following season and on over-wintering carbohydrate was also measured.

Materials and Methods

Trial 1

Site

A commercial block of Pinot Noir (clone 2051) vines planted in 1994 and with rows orientated east to west was selected in the Bay of Fires Vineyard at Pipers Brook, Tasmania. Rows selected for use in this trial were located near the middle of the block with sufficient buffering to avoid edge effects. The vines had an intra row spacing of 1.6m and an inter row spacing of 2.36m.

The vines had been regularly pruned to a 4 cane, Scott Henry system, with two fruiting wires, one at 1.0 and the other at 1.15m. The vines were pruned to one 2 bud basal spur for new cane growth, and 40 potentially fruitful buds on the 4 canes. Vineyard records indicated an average yield of 10 tonnes per hectare per annum.

Weather data in the three seasons of the trial, were obtained from the nearest Bureau of Meteorology station at Ti Tree Bend and are as shown in Table 4.2.

Table 4.2 Weather data from Ti Tree Bend for the three seasons of Trial 1.

Vintage	Mean maximum temperature during pre-anthesis (°C)	Total rainfall during pre-anthesis (mm)	Sunshine hours during pre-anthesis
2002	18.9	62	6.8
2003	20.4	32	9.8
2004	20.9	5.9	10.1

Treatments and experimental design

Four pruning treatments were applied to single vine plots in a five replicate, randomised complete block design. The pruning treatments (shown in plates 4.1 to 4.4) simply involved a sequential reduction in the number of canes from four in the commercial system to 3, 2 or 1. The conversion from the commercial pruning system took place in winter of 2001 and the same pruning treatments were then continued unchanged through 2002 and 2003.



Plate 4.1 Treatment 1, with one 10 bud cane trained to the east (position 1), plus a 2 bud spur.



Plate 4.2 Treatment 2, with two 10 bud canes trained to the east (position 1 & 2), plus a 2 bud spur.



Plate 4.3 Treatment 3, with three 10 bud canes, 2 trained to the east (positions 1 & 2) and 1 to the west (position 3), and a 2 bud spur.



Plate 4.4 Treatment 4, with four 10 bud canes, 2 trained to the east (positions 1 & 2) and 2 to the west (positions 3 & 4), and a two bud spur.

Measurements

Measurements were taken in each of the 2002, 2003 and 2004 cropping seasons. At the start of flowering, bunch number was recorded. At commercial harvest all bunches were collected from each cane and any fruit rising from the spur were collected and retained separately. Harvested fruit was placed in sealed plastic bags and stored at -18°C . Bunches were later thawed, counted to determine bunch number and weighed to determine bunch weight. Berries were then counted and mean berry weight per vine calculated. Loose berries were sieved to determine the proportion of small (≤ 5 mm) and large berries (≥ 6 mm). These sizes were chosen to separate sizes according to the berry size differential generally termed “hen and chickens”. The number of shoots that developed from the buds laid down was recorded after leaf-fall. In each year of the trial, trunk circumference was measured on each treated vine, 20 cm below the crown, trunk cross-sectional area calculated, and pruning weights and stem lengths recorded.

Fruit was used to test pruning effects on fruit quality in 2003. After weighing and size grading for yield component analysis as described above, twenty berries from each treatment replicate were randomly selected then stored at -18°C until required for quality analysis.

A single 5mm disc was excised from the side of each berry and the 20 discs for each treatment replicate combined and weighed. The skin discs were then freeze dried to constant weight in a Dynavac freeze drier (450-050) for 24 hours before being ground using a stainless steel mortar and pestle. The ground skins were then

extracted with 10mL of cold 50% methanol, adjusted to pH1.0 and centrifuged at 9500 rpm for 20 minutes at 15°C. The supernatant was decanted and the precipitate extracted once more in the same manner and the supernatants combined. The supernatants were then made up to a final volume of 50mL and filtered through a membrane filter (0.45µm). The total anthocyanins were assayed by measuring absorbance at 520nm using a Unico™ 1100RS spectrophotometer (Mazza *et al.*, 1999). Absorbance was then corrected according to the dry weight of tissue used in the extraction (Hunter and de La Harpe, 1987).

To confirm that Pinot Noir only contained nonacylated anthocyanins (Mazza, 1999), analysis of anthocyanin in grape skin extracts was performed using a Waters Alliance 2690 HPLC system equipped with a Waters 996 photodiode array detector. Separation was achieved on a Waters Xterra MS C18 column (5µm, 2.1 x 150 mm). A flow rate of 0.3 mL/min was used. Solvent A was methanol, and solvent B was 5% (v/v) formic acid in water. For the skin extracts the following solvent gradient was used; 20: 80 for 5 mins then to 40:60 at 23 mins. A 20µL sample was injected directly into the HPLC. Wavelengths from 200 - 600nm were monitored, and chromatograms were generated at 520nm.

The remainder of the berries from the bulk fruit sample for each plot were pressed and sieved to leave raw juice. The pH of the juice was then measured using a Denver Instrument Basic pH meter and total soluble solids measured as °Brix using a hand-held refractometer.

Cane samples were collected for the determination of tissue carbohydrate concentration at the time of pruning in June 2002. Each sample consisted of two 1cm segments cut from the centre and apex of cane position one (see Plate 4.1) on each vine. The samples were stored at -18°C until analysed. Frozen cane samples were transferred to screw top vials and freeze-dried using a Dynavac Freeze-Dryer fitted with an Edwards RV3 vacuum pump. After drying, tissue was ground into a fine powder using a metal rasp followed by a Glen Creston micro hammer mill fitted with a 1mm sieve.

Carbohydrate Analysis

Extraction and separation of carbohydrates from the freeze dried cane tissue was based on the method described by Lambrechts *et al* (1994). 100mg of each freeze dried sample was placed in centrifuge tubes and 5mL of 80% ethanol was added. Each tube was incubated at 60°C for 1 hour. The samples were then centrifuged for 10 minutes at 3000 rpm at 16°C, using a Beckman model J2-21 centrifuge (Beckman Instruments, Fullerton, USA). The supernatant was removed and stored and the pellet extracted twice more in the same way. The supernatants were then combined and stored in the freezer at -18°C for soluble sugar assay. The pellets were freeze-dried and later analysed for starch.

The combined supernatant from the initial extraction was filtered and the ethanol removed using a rotary vacuum concentrator. The residue was made up to 2.5mL with distilled water and washed twice with chloroform (5:8 v/v). The water phase was used to determine soluble glucose, sucrose and fructose fractions using HPLC -

MS. The column was a Waters High Performance Carbohydrate Cartridge, 4.6mm x 250mm, fitted with a guard cartridge of the same material. The mobile phase was 75% methanol/25% water, isocratic at 1.2mLs per minute. Sugars were detected by Atmospheric Pressure Chemical Ionisation mass spectrometry on a Finnigan LCQ ion trap MS. Retention times were determined from standard solutions, and calibration curves for response were generated over the expected concentration range.

Sugars were detected using negative ion adducts formed by post column infusion of 20 microlitres/minute of 5% formic acid in water. For the monosaccharides tandem MS was used, with the M^+ formate anion at 225.3 being isolated, activated at 25% collision energy, and the subsequent daughter at m/z 179 being further isolated and activated at 25% collision energy with the final products at m/z 89, 119, 131 and 143 being used for quantitation. For sucrose, quantitation was achieved by selected ion monitoring of the M^+ formate anion at 387.3.

Starch levels were determined enzymatically using a total starch assay kit (Megazyme Pty Ltd, Australia). The freeze-dried pellet retained from the soluble sugar assay was weighed into a 20mL glass test tube with a 10.0mL volumetric marking. 3mL of thermostable α -amylase in MOPS buffer (50mM, pH 7.0) was added and mixed using a Chiltern Scientific Vortex mixer, prior to incubating at 100°C for 6 minutes, vortexing vigorously again every 2 minutes. The tubes were then transferred into a 50°C water bath and 4mL of sodium acetate buffer (200mM, pH 4.5) added followed by 0.1mL of amyloglucosidase. The contents of the tube

were vortexed and incubated at 50°C for 30 minutes. After incubation the volume of the tube contents was adjusted to the 10.0mL volumetric mark using distilled water. The tube was then vortexed and centrifuged at 3000rpm for 10 minutes. Duplicate aliquots of the tube contents (0.1mL) were transferred to the bottom of glass test tubes (16 x 100mm). 3.0 mL of GOPOD reagent (glucose oxidase, >12 000units/L; Peroxidase, >650units/L; 4-Aminoantipyrine, 0.4mM; Megazyme Australia Pty. Ltd.) was added to each tube. The tubes were incubated at 50°C for 20 minutes and the absorbance of each sample, including glucose standards were read against the reagent blank at 510nm with a spectrophotometer. Calculation of percentage starch and mg/g dry weight starch in the sample was achieved using the following equations:

$$\% \text{Starch} = \Delta E \times F \times 100 \times 1/1000 \times 100/W \times 162/180$$

where:

ΔE = absorbance of sample read against the reagent blank

F = $100(\mu\text{g of glucose})/\text{absorbance for } 100\mu\text{g of glucose}$

100 = volume correction

$1/1000$ = conversion from μg to mg

W = the weight (in mg) of tissue analysed

$100/W$ = conversion to percentage dry weight

$162/180$ = adjustment from free glucose to anhydro glucose

$$\% \text{Starch} = \Delta E \times F/W \times 9$$

$$\text{Starch (mg/g)} = \% \text{ Starch} \times 10$$

Data Analysis

All results were normally distributed and untransformed data were analysed using a repeated measures ANOVA in the general linear models package of SPSS. Means were compared using least significant difference (LSD) calculated at $P=0.05$ after the method of Steel and Torrie (1980).

For grape quality analysis there was no change in the 4 treatment by 5 replicate experimental design, but samples were taken in one year only (2003) and repeated measures analysis was not required. Data was normally distributed and was analysed using the univariate analysis of variance in the general linear models package of SPSS. Univariate linear regressions of treatment means for absorbance and pH were plotted against treatment means for the various yield components and vegetative growth also using SPSS. A correlation analysis was carried out for all independent variables.

Trial 2

Site

A canopy management combined with a fruit thinning trial was established on Pinot Noir vines (clone 2051) in their third commercially bearing year in fruiting season 2003. The vines were located in Meadowbank Vineyard at Cambridge in Southern Tasmania. In row vine spacing was 1.5m by 2.1m between rows, giving a vine density of 3175 vines/ha. Vines had been pruned to two canes (20 buds) and three canopy management treatments were applied; 20 buds as in the original pruning plus

a, medium canopy of 30 buds and a heavy canopy of 40 buds. The canopy structure for these treatments was similar to Trial 1 with 2, 3 or 4 canes respectively or some canes left longer where the base number of canes did not give enough buds.

Weather data for the critical periods of the two seasons of the trial, taken from the nearest Bureau of Meteorology station at Hobart Airport were as shown in table 4.3.

Table 4.3 Weather data from Hobart Airport for the two seasons of Trial 2.

Vintage	Mean maximum temperature during pre-anthesis (°C)	Total rainfall during pre-anthesis (mm)	Sunshine hours during pre-anthesis
2003	20.7	13	8.4
2004	20.3	2.2	9.7

Treatments

The fruit thinning treatments involved thinning to target fruit yields of 6 (industry standard), 10 and 14 tonnes/ha. Target yields were based on planting density and an expected mean bunch weight of 125 g, requiring bunches to be thinned to 14, 25 and 34 bunches per vine respectively. Bunches were selected for removal using a random number table and were thinned at the start of veraison. Bunch number before thinning was recorded in both years. The same pruning and fruit load treatments were imposed on the same vines for the two consecutive years (2003 and 2004).

Measurements, Experimental Design and Analysis

The trial design was a three pruning treatments by three crop loads factorial with five replicates in a randomised complete block design, continued over two years.

Treatments were applied to single buffered vine plots. At harvest, yield data were collected, as for Trial 1. Analysis was similar to Trial 1, using repeated measures ANOVA in the general linear models package of SPSS. In the first year bunch number prior to thinning was analysed as a randomised block design with only the pruning treatments, and analysed the same as for other parameters in the second season. Again data was normally distributed and means were compared using the LSD at $P=0.05$, calculated as described earlier.

Results

Trial 1

Total yield per vine varied with the number of buds left at pruning. Mean fruit yield across years increased significantly ($P<0.05$) with increased bud numbers. There was also a significant ($P<0.05$) year effect (Figure 4.1) with the 2002 yield significantly lower than in the two subsequent years. The yield increase was not proportional to the number of buds remaining, with a two-fold increase in total yield accompanying the four fold increase in bud number from 10 to 40 buds in each of the three years.

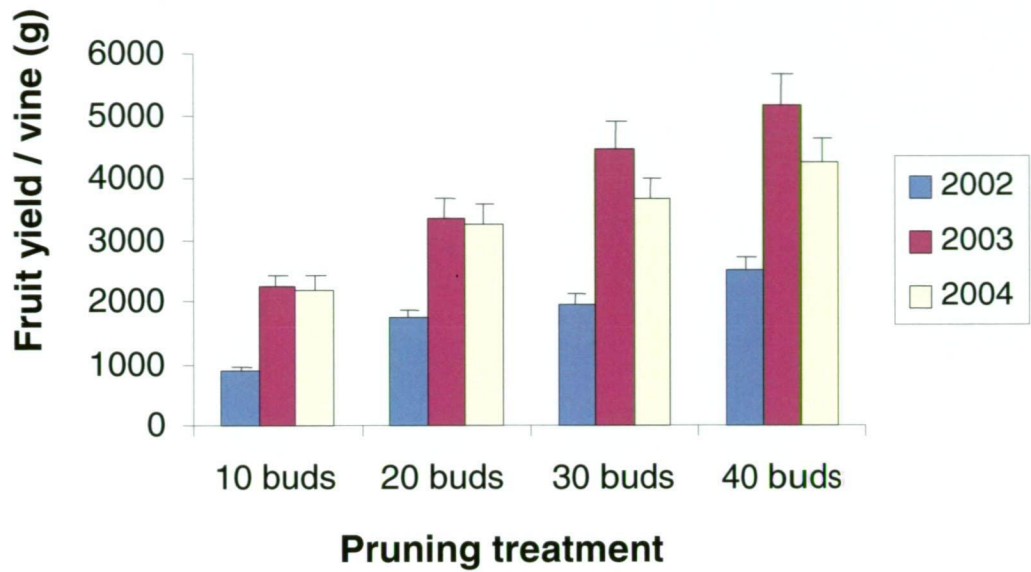


Figure 4.1 Total fruit yield per vine at harvest for the four pruning treatments in 2002, 2003 and 2004. The error bars presented represent standard errors of the means.

For each of the yield components measured, (bunch number, bunch weight, berry number and berry weight), there was a significant ($P<0.001$) year effect but there was no interaction ($P>0.05$) between year and treatments. Overall there was a significant treatment effect ($P<0.001$) on bunch number, but there were no other treatment effects (Table 4.4).

Total bunch number increased with the number of buds (figure 4.2a). However the number of bunches per bud decreased with increasing number of buds. Bunch

number was markedly lower in 2003 and 2004 compared with the first year, 2002.

For example, the 40 bud treatment decreased from a mean of 58 bunches in 2002 to 38 bunches in 2003 and 32 bunches in 2004.

Although there was a significant increase in the number of bunches per vine with increase in the number of buds remaining after pruning, the increase was not proportional to the number of buds imposed. That is, when the number of bunches were expressed on a per bud basis, there was no significant difference ($P>0.05$) between the 10 bud (1.6 bunches/bud) and 20 bud (1.5 bunches/bud) treatments, but there was a significant reduction ($P<0.05$) to 1.2 bunches/bud for the 30 bud treatment and a further reduction to 1.0 bunches/bud for the 40 bud treatment.

There was not a significant ($P>0.05$) treatment effect on mean bunch weight, but there was a significant ($P<0.05$) year effect. In 2002 the number of bunches were high but mean bunch weight was low due to both a low number of berries per bunch and a low mean berry weight. In 2003 and 2004 the reverse was evident with low

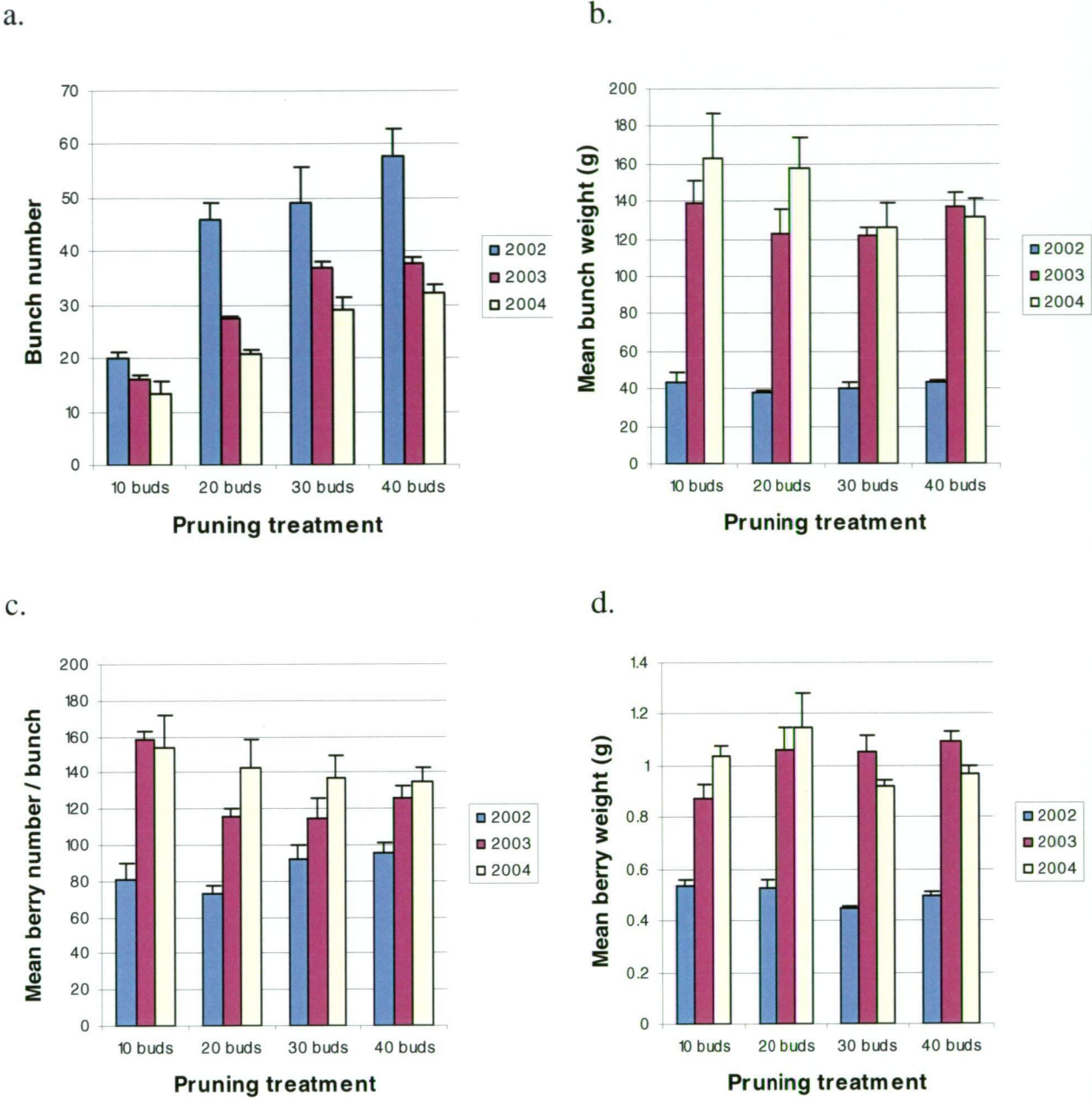


Fig 4.2 Fruit yield components at harvest for the four pruning treatments in the three vintages of 2002, 2003 and 2004. (a.) Bunch number, (b.) Bunch weight (grams), (c.) Berry number, (d.) Berry weight (grams). There was no significant interaction between treatment and years for any of these yield components. The error bars presented represent standard errors of the means. Means across years are shown in table 4.4.

number of bunches and high berry numbers and berry weights. Mean bunch weights were more than three times greater in 2003 and 2004 compared with 2002 for each of the four pruning treatments. Mean berry weight was not significantly effected by pruning ($P < 0.05$) (Table 4.4).

Table 4.4 Bunch number and weight, and berry number and weight as effected by pruning treatments ranging from 10 buds per vine to 40 buds per vine. Figures presented are the means for the three years. Figures with the same superscript were not significantly different based on the LSD ($P = 0.05$).

Pruning Treatment	Mean bunch no. per vine	Mean bunch weight	Mean berry no. per bunch	Mean berry weight
10 buds	16.38 ^a	119.14	130.16	0.87
20 buds	31.15 ^b	111.31	113.46	0.93
30 buds	38.27 ^{bc}	95.91	117.34	0.79
40 buds	42.60 ^c	105.87	119.31	0.86
LSD ($p = 0.05$)	10.62	ns	ns	ns

Pruning weights were the same for all treatments and although trunk cross-sectional area increased over the three years there was no treatment effect, as shown in Table 4.5.

Budburst data was heavily skewed and analysis of variance was not carried out. Results are given in Table 4.6 and there was no apparent pattern between years or treatments. Treatment means across years was similar for all treatments with around 10 to 20 percent of buds failing to develop. There was a significant interaction ($P<0.05$) between years and treatments for the mean number of bunches per viable bud with greater bunch numbers generally resulting in fewer bunches per viable bud. With the 10 bud treatment there was no significant difference in bunch number per bud across the three years, with the 20 and 40 bud treatments showing a marked decline from 2002 to 2003.

Table 4.5 Pruning treatments and vine characteristics of the treatment vines (Pinot Noir vines clone 2051), at Bay of Fires Vineyard, Tasmania. There were no significant differences between pruning weights and no significant treatment effects on trunk cross-sectional area.

Treatment	2002		2003		2004	
	Trunk cross-sectional area	Pruning weight (kg)	Trunk cross-sectional area	Pruning weight (kg)	Trunk cross-sectional area	Pruning weight (kg)
1	8.5	1.4	12.6	1.3	15.6	1.5
2	10.6	1.2	13.4	1.2	16.7	1.3
3	9.0	1.5	11.9	1.4	14.7	1.6
4	10.5	1.5	12.9	1.4	15.3	1.6

Table 4.6 Percent budburst and bunches per viable bud for the four pruning treatments over the three experimental years. Budburst was not statistically analysed (see text). There was a significant interaction between years and treatment for bunch data, LSD ($P=0.05$) = 0.33.

Treatment	2002		2003		2004	
	Bud Burst	Bunches per viable bud	Bud Burst	Bunches per viable bud	Bud Burst	Bunches per viable bud
1	100	2.01	92	1.80	78	2.06
2	88	2.38	85	1.63	83	1.65
3	85	1.26	86	1.01	80	1.61
4	87	1.83	86	1.09	87	1.09

There was a significant pruning treatment ($P<0.05$) effect on the starch content of winter canes as shown in Table 4.7. Canopies with 10 and 20 buds per vine had a starch content significantly higher than the 30 and 40 bud canopy treatments. There was no difference in starch content between the 30 and 40 bud treatments. Starch as a percentage of the total extracted carbohydrate was also significantly higher ($P<0.05$) in the 10 than the 20 bud treatments.

Soluble carbohydrate concentrations (Table 4.7) were not effected by pruning treatment, with each of fructose, glucose and sucrose showing no significant differences ($P>0.05$) between pruning treatments.

Table 4.7 Level of starch and soluble sugars (mg/g) on a dry weight basis in the cane during dormancy as affected by pruning treatment ranging from 10 buds per vine to 40 buds per vine. Figures with the same superscript were not significantly different based on the LSD ($P=0.05$).

Pruning Treatment	Starch	Fructose	Glucose	Sucrose
10 buds	40.3 ^a	8.69	11.37	4.80
20 buds	52.7 ^b	9.39	13.00	5.11
30 buds	8.1 ^c	8.50	11.35	5.85
40 buds	8.2 ^c	8.04	18.04	4.17
LSD ($p=0.05$)	11.5	ns	ns	ns

There was a marked variation in percent bud break in the 10 bud treatment from year to year, however there were too many zeros to do a valid statistical analysis. With higher bud numbers (20-40) however, there was less year to year variability falling to a minimum with 40 nodes.

HPLC analysis of the methanol extract revealed the presence of nonacylated anthocyanins (delphinidin 3-monoglucoside-*p*-coumarate, cyaniding 3-monoglucoside-*p*-coumarate, petunidin 3-monoglucoside-*p*-coumarate, peonidin 3-monoglucoside-*p*-coumarate, malvidin 3-monoglucoside-*p*-coumarate) (Fig 4.3). No acylated anthocyanins were detected. The colourmetric assay showed a significant

($P < 0.05$) treatment effect on both pH and absorbance, but not on sugar content ($^{\circ}\text{Brix}$) (Table 4.8). As the number of buds retained per vine increased from 10 to 30, there was a significant ($P < 0.05$) increase in absorbance but in the 40 bud treatment, the absorbance decreased to a level not significantly different ($P > 0.05$) from the twenty bud treatment.

The pH of the ten and twenty bud treatments, was significantly ($P < 0.05$) higher than the 30 and 40 bud treatments. There was no significant ($P > 0.05$) difference in pH between the 10 and 20 bud treatments, nor between the 30 and 40 bud treatments.

Fig 4.3 HPLC chromatogram of anthocyanins in Pinot Noir grape skin extracts monitored at 520nm. The peaks and corresponding non-acylated anthocyanins shown were as identified by Mazza *et al* (1999).

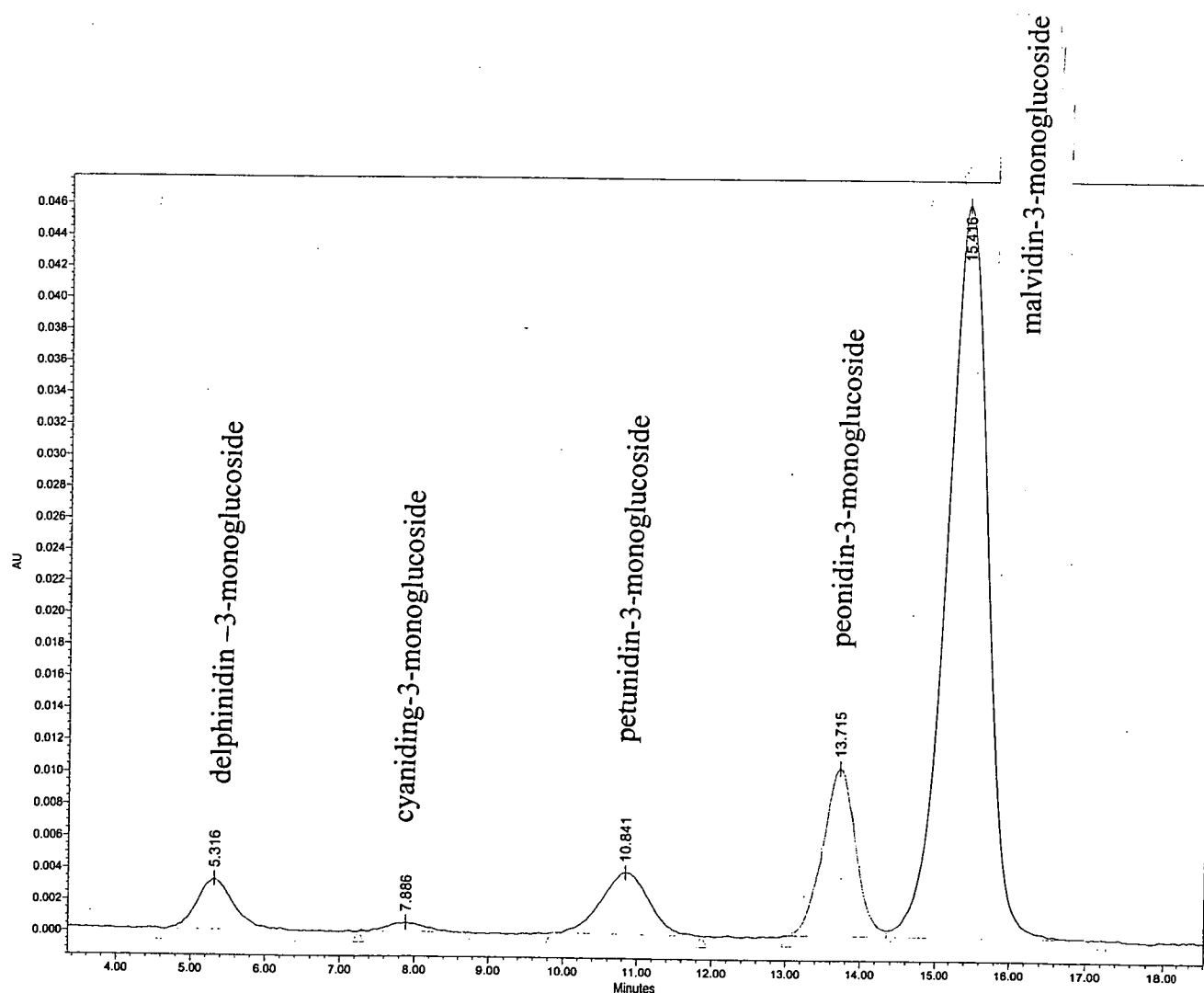


Table 4.8 Absorbance (520 nm), pH and sugar content (° Brix) of fruit in relation to pruning treatment. Figures with the same superscript were not significantly different based on the LSD (P=0.05).

	Absorbance (520 nm)	pH	° Brix
10 bud treatment	0.48 ^a	3.46 ^a	20.68
20 bud treatment	0.69 ^b	3.48 ^a	20.53
30 bud treatment	0.82 ^c	3.33 ^b	19.74
40 bud treatment	0.65 ^b	3.33 ^b	20.28
LSD (p=0.05)	0.099	0.100	ns

There was a significant ($P < 0.001$) negative correlation ($r^2 = 0.64$) between berry number per bunch and absorbance (Fig 4.9). Other correlations between quality and yield or growth parameters were not significant and therefore results are not shown. There was also a significant ($P < 0.05$) positive correlation between pH and °Brix (Fig 4.10).

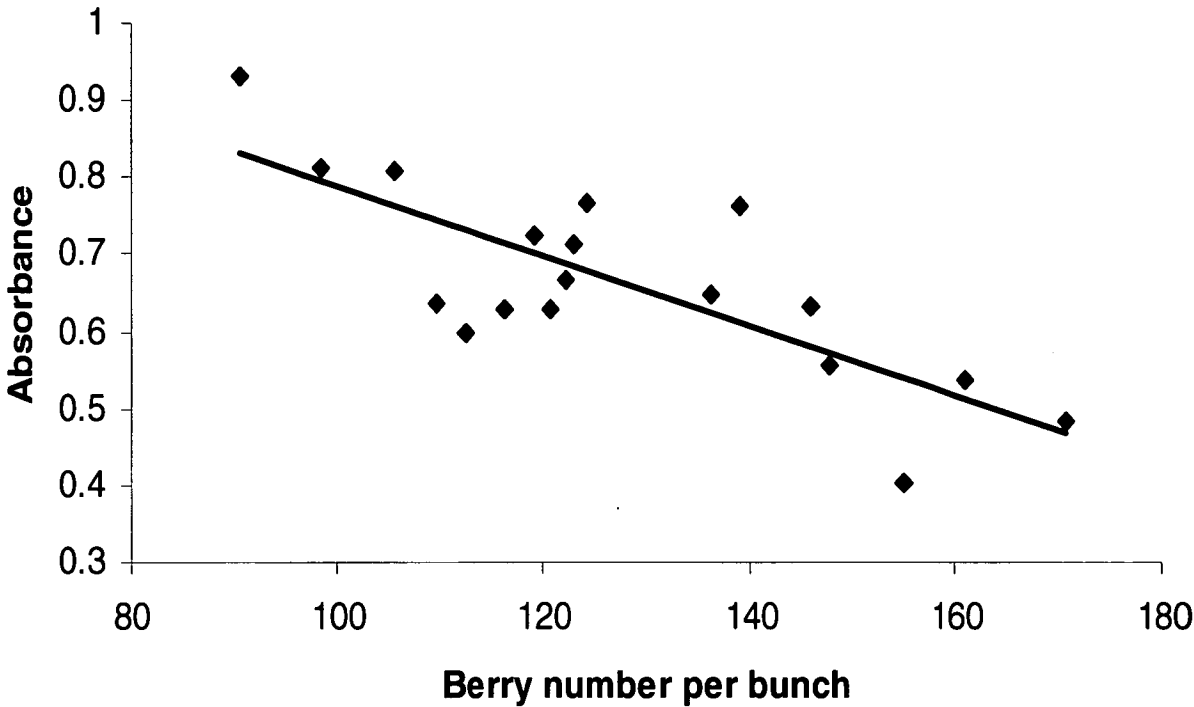


Figure 4.9 Relationship between berry number per bunch and absorbance (520nm) in 2003.

Liner regression where $y = -0.0045x + 1.2418$, and $R^2 = 0.6098$.

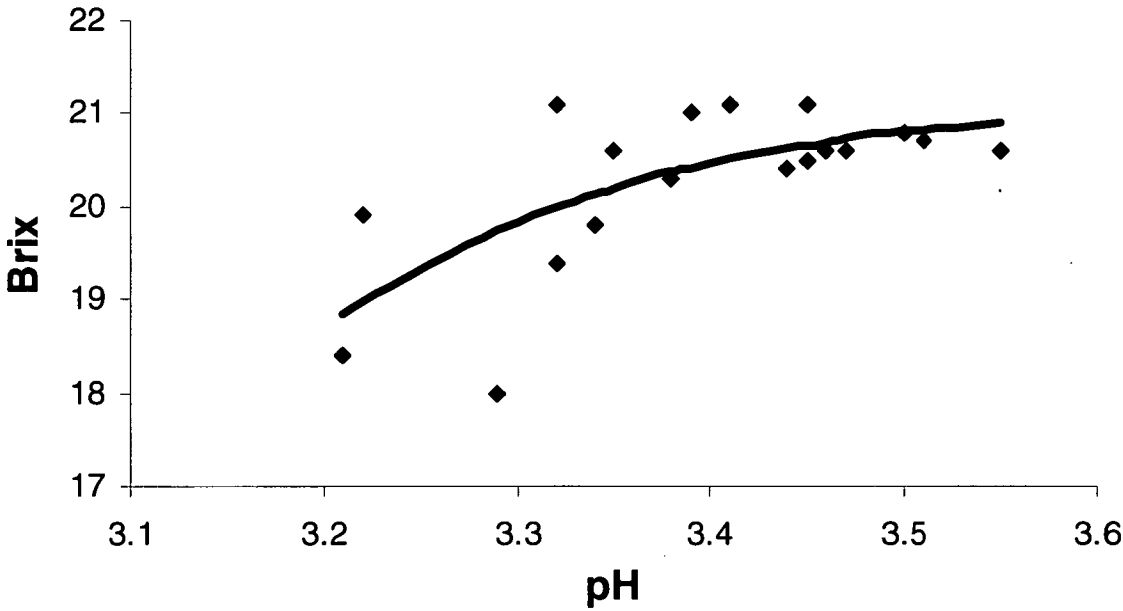


Figure 4.10 Relationship between °Brix and pH in 2003. $R^2 = 0.47$.

Trial 2

A summary of the fruit yields and yield components in response to the three cane pruning by three fruit thinning factorial experiment conducted at trial site 2 in Southern Tasmania is shown in Table 4.11. There was no significant interaction between bunch number and pruning regime in the first year (2003). There was a significant pruning effect ($P < 0.05$) on bunch number before fruit thinning (Table 4.12) with bunch number being significantly higher in the 40 bud treatment with 55.9 bunches per vine, as compared with 37.0 and 36.7 in the 20 and 30 bud treatments respectively. The number of bunches per bud was also significantly affected by pruning treatment the 20 bud treatment being significantly higher than the 30 and 40 bud treatments. In the second year (2004) there was again a significant pruning effect (Table 4.13) as well as a significant fruit thinning effect (Table 4.14) but no pruning by thinning interaction. In 2004 the bunch number for the 30 and 40 bud treatments were not significantly different from each other but were significantly ($P < 0.05$) higher than the 20 bud treatment. The number of bunches per bud decreased significantly as the number of buds increased. The thinning treatment caused a significant decrease in bunch number between the 6 tonnes per hectare and 10 tonnes per hectare fruit thinning treatments. The bunch number of the 14 tonnes per hectare treatment was not significantly different to the 10 tonnes per hectare treatment.

In 2004 vines that were thinned to a target 6 tonnes/ha had a significantly ($P < 0.05$) greater number of bunches than the 10 and 14 tonnes/ha target yield treatments (Table 4.14).

Table 4.11 Fruit yield thinning treatments for 2003 and 2004, showing target yield and actual mean yield.

Pruning Treatment	Target yield (t/ha) imposed by bunch thinning at veraison	2003		2004	
		Actual mean bunch wt (g)	Actual tonnes/ha	Actual mean bunch wt (g)	Actual tonnes/ha
20 buds	6	101.91	4.5	170.84	7.59
	10	115.97	9.2	151.73	12.04
	14	87	9.3	144.12	15.56
30 buds	6	103.49	4.6	149.93	6.66
	10	102.03	8.1	165.03	13.10
	14	103.86	11.2	161.12	17.39
40 buds	6	103.74	4.6	143.21	6.37
	10	95.21	7.15	158.54	12.58
	14	89.52	9.6	145.88	15.75
LSD (P=0.05)		ns		ns	

Table 4.12 Bunch number in response to pruning treatment 2003 measured before thinning treatment.

Pruning Treatment	Bunch number	Bunches per bud
20 buds	37.9 ^a	1.9 ^a
30 buds	36.7 ^a	1.2 ^b
40 buds	55.9 ^b	1.4 ^c
LSD (p=0.05)	6.13	0.17

Table 4.13 Bunch number in response to pruning treatments 2004 measured before fruit thinning treatment.

Pruning Treatment	Bunch number	Bunches per bud
20 buds	32.3 ^a	1.61 ^a
30 buds	40.9 ^b	1.36 ^b
40 buds	43.7 ^b	1.09 ^c
LSD (p=0.05)	6.02	0.27

Table 4.14 Bunch number in 2004 measured before fruit thinning treatment.

Target Fruit Load	Bunch Number
6 tonnes/ha	46.7 ^a
10 tonnes/ha	36.6 ^b
14 tonnes/ha	33.6 ^b
LSD (p=0.05)	6.02

For each of the yield components measured at harvest, bunch weight, berry number and berry weight, there was a significant year effect ($P < 0.05$) but no significant interaction between years ($P > 0.05$) and treatments. There was no interaction between winter cane pruning and fruit thinning treatments ($P > 0.05$). Results for fruit thinning treatments of each for the means across the two years are shown in Table 4.15 and for pruning treatment Table 4.16.

Table 4.15 Bunch weight and berry number and weight, in response to fruit load treatments in trial 2 as means across years. Figures with the same superscript were not significantly different based on the LSD ($P = 0.05$).

Target Fruit Load	Berry number per bunch	Mean berry weight (g)	Mean bunch weight (g)
6 tonnes/ha	138.05 ^a	0.94	128.85
10 tonnes/ha	138.77 ^a	0.96	131.42
14 tonnes/ha	124.71 ^b	0.98	121.92
LSD ($p = 0.05$)	12.99	ns	ns

Table 4.16 Bunch weight and berry number and weight, in response to pruning treatments in trial 2. Figures presented are means across years.

Pruning Treatment	Berry number per bunch	Mean berry weight (g)	Mean bunch weight (g)
20 buds	133.37	0.97	128.6
30 buds	138.19	0.96	130.91
40 buds	135.36	0.95	122.69
LSD (p=0.05)	ns	ns	ns

Results for the two years of both the pruning and thinning treatments are given in Appendix 4 as mean, berry number, berry weight, and bunch weight.

Discussion

A notable feature of the yield results in Trial 1 (Fig 4.1) was the marked year effect compared with treatment effects. To determine the proportion of variability explained by pruning treatment, an assessment of relative contributions of year and treatment effects was carried out using sum of squares in the ANOVA. The treatment effect accounted for 44% of the variability in bunch number but 2% or less of the

variability for bunch weight, berry number and berry weight. Because bunch number is the primary determinant of yield (Martin *et al.*, 2000) the fact that treatment effect accounted for 44% of the variability in total yield is consistent with this hypothesis. The year effect accounted for 32% of bunch number variability, 66% for bunch weight, and 40% and 70% for berry number and berry weight respectively. Thus, the environment had a moderate effect on variability associated with factors determined prior to flowering and a larger effect post flowering on berry weight and bunch weight. This is reflected in the lack of significant treatment effects in these parameters but significant year effects.

Low bunch weights, berry number per bunch and berry weights (Fig 4.2) may be due to the cool, wet and cloudy flowering conditions experienced in the 2002 vintage (Table 4.2). Further, the results agree with the conclusions from Chapter 2 that the predominant effect of seasonal conditions on yield appears to be via bunch size.

The effect of the pruning regime in Trial 1 on bunch number was unexpected. Industry generally accepts that Pinot Noir produces two bunches/bud and bunch number per bud would be expected to remain relatively constant as node number increased rather than the significant decrease observed here. The budburst and mean bunches per viable bud data indicates that the effect on bunch number was almost certainly due to variation in the number of bunches developing from viable buds.

The results from the present study concur with the findings of Clingeleffer and Sommer (1994), who found a similar trend in a pruning experiment on Cabernet

Sauvignon vines ranging from 2 buds to 14 buds per vine. Bunches per bud decreased from 1.74 at the 2 bud treatment to 0.83 at the 14 bud treatment. Their results also demonstrated within vine control of budburst and showed the strong influence of number of buds on percentage budburst. Unfortunately the authors did not comment on numbers of bunches per viable bud.

Trials conducted at Lincoln College by Jackson and Steans (1983-4) suggested that yield should increase with bud number up to a level likely to be specific for that cultivar and district. Above this level, a further increase in bud number would be expected to have a limited effect on yield since the photosynthetic ability of the canopy would be reaching capacity, or the potential of the soil and roots to provide further water and nutrients would be nearing exhaustion. Chardonnay and Gewurztraminer vines used in their study showed no peak in production, with yield paralleling bud numbers up to the treatment maximum of 84 buds per metre of row. Sauvignon Blanc yields increased until bud numbers reached 48 per metre, after which only marginal increases occurred. Muller Thurgau yields levelled off at about 40 buds per metre and Cabernet Sauvignon showed no response to increased buds per metre. The authors suggested that Cabernet Sauvignon may have a low yield threshold which cannot be increased by increasing buds above a relatively low number. Results from the present study suggest that Pinot Noir vines of the clone 2051 may also have a low to medium threshold, with yield levelling off after 30 buds.

The results obtained in the carbohydrate analysis confirmed that bud number determined at pruning had an effect on the starch fraction of stored carbohydrate in the following winter, but had no effect on soluble carbohydrates. Values for total soluble carbohydrates were consistent with results of Sommer and Clingeleffer (1995), whose figures were also within the range reported by other authors (Ruhl and Alleweldt, 1990; Ruhl and Clingeleffer, 1993). Starch levels were also comparable with the Sommer and Clingeleffer study. Generally plants would be expected to maintain near constant soluble carbohydrate levels and hence cell osmotic potential, using reserve starch as an osmotic buffer (Priestley, 1962).

Sommer and Clingeleffer (1995), despite showing a similar annual course in the total concentration of carbohydrate in the wood of two different pruning systems, found almost twice the carbohydrate storage in minimally pruned vines as compared with cane pruned vines, because of a much larger wood mass in the former. The conclusion was reached that the size of the vine was a more important factor in determining the storage of carbohydrate in the vine than the concentration of carbohydrate in the wood tissue. In the present study, there was no significant difference in vine size as determined by trunk cross-sectional area or pruning weight response to treatment, therefore carbohydrate concentration differences reflect real differences in total reserves in response to treatment.

The higher reserve carbohydrate levels in the 10 and 20 bud canes compared with the 30 and 40 bud canes corresponds with higher bunches per bud overall and per viable bud in these treatments the following year in 2003.

Petrie *et al* (2000) showed that despite uncropped vines having a larger leaf area compared with cropped vines, uncropped vines did not produce a higher total dry weight than the respective cropped vines and therefore concluded that in uncropped vines carbon assimilation was sink-limited. Flore and Lakso (1989) suggested that sink limitation might have been caused by end-product inhibition of photosynthesis, when sugars are not utilized as rapidly in the shoots and roots as they are synthesized by the leaves. In the cropped vines, similar photosynthetic rates were measured in all cropped treatments at post veraison, and were thus suggested to be carbohydrate source limited (Petrie *et al.*, 2000b). In the present study starch reserves showed a marked increase when fruit load was decreased with bud number reduction from 30 to 20, but there was no further increase with a further decrease in bud number. This suggests possible source limitation of photosynthesis with low crop load, agreeing with Petrie *et al.*, (2000a).

Edson *et al* (1993) found that while management practices can influence where the carbohydrates produced by the vine are partitioned (fruit or vegetative growth), it is difficult to increase the absolute amount of dry matter produced through management practices. Bennett *et al* (2000) reported that reduced over-wintering carbohydrates due to defoliation of Chardonnay vines caused a reduction in both inflorescence number per bud (shoot) and the flower number per inflorescence. The authors suggested that vines should be managed both for the current crop and for subsequent crops in order to maintain sufficient carbohydrate reserves for balanced growth and cropping from year to year. This result appears to agree with the results from the

present trial, where less bunches per bud were apparent in treatments with lower overwintering starch concentrations. The overall yield for treatments 3 and 4 with 30 and 40 buds respectively was significantly less in 2004 compared with 2003. This result may be due to the vines with 30 and 40 buds having significantly lower reserve starch levels and hence being more dependent on current photosynthate at flower initiation than the vines with smaller canopies of 10 and 20 buds.

The results of both trials demonstrate that the number of buds remaining after pruning and fruit thinning significantly affect yield components. Bunch numbers and bunch weights were both significantly different in the year following the treatments being applied, thus suggesting that both the pruning and fruit thinning treatments in the current year impacted on inflorescence initiation and differentiation for the following year. The significant effect on bunch number prior to fruit thinning in Trial 2 was consistent with the results of Trial 1. In 2003, where the thinning treatments had not yet been imposed, bunch number per vine was significantly higher in the 40 bud treatment than the 20 and 30 bud treatments. In 2004 bunch number was lower for the 40 bud pruning treatment than in 2003, dropping from 55.9 to 43.7, while the 20 and 30 bud treatments did not exhibit this drop in bunch number in 2004. This decrease in bunch number at the 40 bud treatment may be due to a carryover effect from 2003 due to overcropping, consistent with results in Trial 1.

In 2004, the effects of the thinning treatment on bunch number before thinning showed a significantly higher bunch number in the 6 tonnes per hectare treatment than in both the 10 and 14 tonnes per hectare treatments. This also suggests a carry

over effect from 2003 to 2004. At the highest fruit load treatment of 14 tonnes/ha, and 10 tonnes/ha the number of bunches may have been significantly lower due to overcropping in 2003.

The number of berries per bunch was significantly lower at the high fruit load of 14 tonnes/ha than the 6 and 10 tonnes/ha treatments, but this did not correspond with a significant decrease in bunch weight since the bunches with lower numbers of berries had a slightly higher berry weight. This result contrasts with Bravdo (1985) who reported that the number of berries per bunch was not affected by bunch thinning, and bunch weight compensation of the thinned treatments was due mainly to increased berry weight, however the Bravdo study used Cabernet Sauvignon on 110 R rootstock, which may explain the discrepancy in results.

The fact that the pruning treatments in Trial 2 had no significant effect on berry number, weight or bunch weight was consistent with Trial 1, where pruning only affected bunch number.

The seasonal conditions during fruitset and current season growth appear to have a major impact on final yield, as illustrated by the large variability in yield per vine in both trials. The results obtained in Trials 1 and 2 confirm the findings of Chapter 2 that management practices appear to override or at least interact with environmental effects on yield. Pruning regimes in both trials, significantly affected bunch number, the fruit yield component regarded as the primary yield determinant (Martin *et al.*, 2000) and resulted in differences in final fruit yield. The results in Trial 2 suggest

that target yields can be achieved successfully by winter pruning to set bud numbers and bunch thinning after fruitset, and that as long as quality is not negatively affected by greater yields, the possibility exists for growers to use this as a measure for decreasing yield variability between years. Further work is necessary to determine the implications for canopy size and potential fruit shading of employing this strategy. The difficulty for growers using this method appears to be estimating the effect of seasonal conditions on bunch weight.

The pruning treatments imposed in Trial 1 had a significant impact on two of the three measured aspects of fruit quality. Anthocyanin levels were lower with lower bud number, whilst there was a small but significant decrease in pH at high bud numbers. Importantly however, there was no significant effect on °Brix, suggesting that the treatment effects are directly on the fruit quality parameters measured rather than indirect effects related to delayed fruit maturity at higher crop loads.

These results, suggesting that these aspects of wine quality were enhanced in vines pruned to give higher yields, are contrary to industry management practice, which assumes that quality declines above yields around 6 tonnes/hectare. Whilst there is some research indicating improved quality with lower yields, most seems to conclude that there is no effect, provided differences in maturity are taken into account.

The significant negative correlation between berry number per bunch and absorbance may at least partly explain the relationship between yield and colour. The decrease in

anthocyanin content with increasing berry number suggests that (in this experiment) colour development may be related more to internal shading within the bunch than to internal shading within the canopy. In the present trial, there was no treatment effect on pruning weight suggesting that canopy shading was not influenced by treatment. Thus the apparent relationship between bunch size (as berry number) and pigment development requires further investigation.

Rojas-Lara and Morrison (1989) reported that anthocyanin accumulation in the fruit was affected more by cluster shading than by leaf shading. Haselgrove *et al* (2000) reported that anthocyanin metabolism responds to changes in both light and temperature conditions. Responding to the findings of Dokoozlian (1990) who found that Pinot Noir berries showed maximum colour accumulation at low light intensities, of less than 18% ambient photosynthetic photon flux density, Haselgrove (2000) suggested that if light conditions within a canopy are such that the bunches receive sufficient light of moderate intensity, then light is not necessarily a limiting factor for anthocyanin synthesis. Overall, these reports appear to support the results obtained in the present trial and indicate that internal shading in the canopy is not a major determinant of fruit colour.

Some care is necessary in interpretation of results showing a canopy shading effect. For example, in an experiment by Hunter and de La Harpe (1987), lower bud numbers led to superior colour expression. However the paper did not clearly establish when pruning treatments were applied. Consequently, in the season when there was a significant effect on colour the result may indeed have reflected a smaller

there were no differences in pruning weight, suggesting that heavy cropping did not influence either total vegetative growth or canopy size.

Results from the fruit thinning trial (Chapter 4) confirmed that it is possible to achieve a target yield by winter pruning to a bud number higher than desirable and bunch thinning to reach the target fruit yield. The largest target fruit load of 14 tonnes/ha had a significantly lower number of berries per bunch than the two smaller treatments, thus suggesting that the larger fruit load affected flower differentiation. Importantly however, year to year variations in yield in the two pruning trials were greater than the treatment effects. Further, treatment effects on bunch number and size were consistent across years, with bunch number per viable bud in lightly loaded vines remaining constant across the three years. There was also little change between the second and third years for the other treatments. There are a few reports of adjustments in bunch number per viable bud noted by (May, 2004) with no clear indication on whether changes are induced by conditions in the current or previous seasons. If current season conditions are involved, results of these two trials confirm the inference from the vineyard and climate / growth cycle observation in Chapter 2. That is, variation in bunch size and possibly bunch number in some seasons, in response to weather conditions are the primary causes of highly variable yields in commercial vineyards in Tasmania. This result particularly the bunch size component has important ramifications for management to both yield and quality targets.

The strong regression between berries per bunch and anthocyanin concentration, found in this study, suggests that the importance of bunch size in wine quality and yield studies should be re-examined. As berry number per bunch increased, the level of anthocyanin in berry skins decreased. Although there was no significant regression between bunch size and pH, the relatively small change in response to treatment may have been insufficient to demonstrate a more general relationship. Further work is clearly required to clarify links between quality and bunch size. Nevertheless, the clear negative regression relating colour to bunch size, the confusing literature on yield / quality relationships and anecdotal evidence relating yield negatively to colour combined with the marked effect of bunch size on yield suggest a critical review of practical bunch thinning and of some research results. It is notable that a review paper by May (2000) noted that intra-bunch shading is likely to influence quality in tight bunched varieties like Pinot Noir. Information on cluster architecture, specifically the number of berries per centimetre of cluster shoulder length or some other measure of cluster compactness are also necessary to further develop the relationship between bunch size and colour. Further detailed fruit analysis and wine chemistry and sensory analysis is also necessary to further our understanding in this area.

Whilst bunch exposure to light has been a dominant theme in canopy structure studies by various authors (Kliewer and Linder, 1970; Smart, 1985; Archer and Strauss, 1989; Jackson and Lombard, 1993; Jeong *et al.*, 2004) the possibility that intra-bunch shading in tightly packed bunches may have an effect on berry exposure has received little attention. Work by Shabala and Wilson (2001) showing that light

canopy in heavier pruned vines. However, in the second season there was no bud number effect. This may have been incorrectly attributed to seasonal conditions, with stronger vegetative growth in the second season leading to little or no differences in canopy size. Unfortunately the authors did not measure pruning weights in either season.

The significant decrease in pH between the 10 / 20 bud and 30 / 40 bud treatments is also not consistent with the literature. In most studies such as the one conducted by Jeong (2004), larger more shaded canopies have a higher pH. Smart, Smith and Winchester (1988) and Archer and Strauss (1989) also reported an increased pH due to within-canopy shading of Cabernet Sauvignon vines. In the present trial there was no significant treatment effect on canopy size, which again suggests that canopy architecture is not limiting the basic fruit quality attributes in these vines.

Overall two basic indicators of wine quality, fruit anthocyanin content and pH indicate that in vines pruned to a crop load in the range 10-40 fruitful buds per vine there is no inverse relationship between bunch size and quality. The negative relationship between bunch size and the quality factors further suggests that any relationship between yield and quality may be an indirect one, resulting from bunch size as a determinant of yield.

Chapter 5 – General Discussion and Conclusions

The result in Chapter 2 confirmed the industry view that yield variability of Pinot Noir grapevines is a major issue in Tasmanian vineyards. Variability was greater than the previously suggested 70 percent figure and in selected vineyards was as high as 150 percent from one year to the next. The lack of any consistent patterns in yield across regions suggested that any weather effect was overridden by local management or climate factors. The rapid development of the industry during this period with a high proportion of young vines in the crop for each year may also have contributed.

In spite of some inconsistencies, results for selected properties showed it is possible that spring weather conditions as either sunshine hours or temperature in the current season and possibly rainfall at fruitset may have a marked impact on final yield. The results of Martin *et al* (2000) in Victoria (Australia) suggested that the largest source of seasonal variation in yield was bunches/vine, typically accounting for 60% of the variation. Bunches per vine is unlikely to vary after spring budburst. Consequently from these results it appears that, although bunches per vine is regarded as a major yield determinant, the extreme local seasonal variability may be due to variability in other yield components. The same authors noted that berries per bunch accounts for 30% of the variability, and as berries per bunch would be expected to respond to conditions in the current season it appeared from this preliminary study that the high variability in Tasmania may be due to berries per bunch or berry size. That is, under Tasmanian conditions, relative contributions of bunch number and size to yield variability, may be reversed compared with previously published results. It is also

important to note that the cultural practice of bunch thinning in Tasmania may be masking the real contribution that bunch number makes to yield variability.

Most vineyards in Tasmania are cane pruned, with a careful selection of the number of fruitful buds retained made at pruning during winter dormancy. This control of potential bunch number is much more precise than in spur, or mechanically pruned vines. Present results (Chapter 4) have shown that, although bunch number per fruitful node decreases with the number of buds, it did not vary significantly between seasons. Thus with strictly controlled bud number and a predictable and stable relationship between bud number and bunches per bud, the experimental results confirm the interpretation of the yield / climate relationship. That is, in the absence of bunch thinning, bunch number varies little from year to year and extreme variability is almost certainly associated with variability in bunch weight. However, the contribution of bunch number to yield variability should not be underestimated. Results suggest that it probably remains the major management factor (as either dormant pruning or bunch thinning) controlling yield. Further, while a marked decrease in bunch number after the first year in Trial 1 Chapter 4, may be related to the change in vine structure in the preceding winter, the possibility that this was not related to other environmental factors cannot be eliminated. Nevertheless, management induced variability may explain why not all of the vineyards examined closely in Chapter 2 demonstrated the postulated effect of weather on yield. Thus, whilst growers use bunch number to manage to a target yield, bunch size will influence actual yields over a broad range around the target.

Changes in bunch number per bud in response to total bud number indicate some control of initiation in response to previous crop probably mediated by carbohydrate levels or associated with endogenous growth substances. Adjustment of bunches per bud after initiation could only occur as a result of some buds failing to break dormancy, but figures showing no significant treatment or year effects on the number of blind buds suggest control at initiation.

Bunch thinning to a target yield at or near veraison is a common practice in Tasmania, so that variability in yield due to bunch number would be expected to be further limited, emphasising the major role of bunch size as the greatest influence on yield. With bunch weight / berry number identified as a potentially critical yield factor, rather than bunch number, fruitset becomes an important factor in managing yield variability.

Despite this sensitivity of yield to fruitset, the literature contains few studies on the method of pollination in grapevines and, even in recent reviews such as May (2004) there is no clear picture of flowering to fruitset process. The results presented in this study demonstrate that Pinot Noir is self-fertile and failure of pollen to germinate until after cap fall suggests that the conditions that promote cap fall are crucial in achieving successful fertilisation and fruitset. These results concur with observation reported in May (2004) that, in cold and rainy weather, flowers may only partially open and irregularly, at temperatures below 15°C - 17°C. It was suggested that if unfavourable weather lasts for two or three days flowers do not open completely, and both pollination and fertilisation are poor, resulting in small bunches (May, 2004).

Drying conditions such as low humidity and high temperatures and high sunlight incidence are thought to aid cap fall. The sunlight influence appears to be indirect, possibly operating as radiant heating causing a rise in temperature of exposed flowers (May, 2004). These same climatic variables during flowering were shown to explain a significant proportion of the yield variability at three vineyard sites within close proximity to weather stations in the present study. Further work is required to show whether delayed cap fall in fact reduces fruitset or simply delays normal pollination, and hence fertilisation, resulting in uneven berry maturity.

Pollen toxicity to fungicide introduced the possibility of reduced fruitset due to routine fungicide applications. This finding, combined with the suggestion that berries per bunch explains a large proportion of bunch-to-bunch, vine-to-vine and season-to-season yield variation suggests that present fungicide programs could be contributing to yield variability. However, the predominant effect of sunshine and or temperature before cap fall in the initial study suggests that the effect may relate to availability of current photosynthate between budburst and cap fall resulting in reduced fruitset in less favourable years.

Canopy manipulation, by altering the number of potentially fruitful buds, significantly affected the number of bunches per bud as well as vine reserve carbohydrate concentrations. The two higher bud number treatments, with significantly lower stored starch concentrations, also had the lowest number of bunches per bud, thus suggesting these vines might have been overcropped and that resultant low carbohydrate levels were influencing inflorescence initiation. Notably,

incident on the berry surface directly influences components of quality emphasises the need to re-examine the current view of fruit exposure. These authors did not, however, consider the potential involvement of intra-bunch shading. Again, the review paper by May (2000) implied that canopy management to increase bunch exposure could have a reduced effect if bunch compactness limits exposure of berries to light under cool climate conditions.

The relationship between yield and vine carbohydrate status also needs to be considered in this context. Leaf plucking to increase fruit exposure may decrease over wintering carbohydrates, and hence yield, in the following year, with few direct advantages in quality. Conversely however, any positive effects may be indirect with reduced carbohydrate status potentially responsible for reduced bunch size in the following season.

Practically, when bunch thinning, growers could selectively remove larger bunches, retaining smaller loose packed ones to maximise quality. Further, whilst bunch thinning to a target yield may be necessary to achieve full fruit maturity, the results suggests that yield effects on quality claimed locally (Farquhar, 2003) and in published literature dating from before Amerine and Winkler (1944) may require some re-examination in their practical application.

Overall, with the possibility of bunch size having a major influence on both yield and quality, management of bunch size assumes primary importance. Present results indicate bud numbers and spring weather conditions as the main controlling

influences. Thus increasing bud numbers during dormant pruning and adjusting bunch thinning regimes to account for spring weather effects on bunch size should be the main components of any revised management strategy. Bunch thinning may also need to be selective, preferentially removing larger tightly packed bunches.

The potential for fungicide application to reduce fruitset also needs further investigation. Whilst avoidance of pollen toxicity will remove a potential contributor to yield variability, timing of sprays to anthesis of early flowers may have the advantage of reducing bunch size, with corresponding increases in quality.

Concluding remarks

Yield and weather records, pruning methods and experimental results suggested that bunch size, is a major yield component contributing to uncontrolled yield variability in Tasmanian Pinot Noir. Weather conditions, particularly spring sunlight and temperature in the current season, appeared to have a greater impact on bunch size (as both fruit number and size) than the number of buds left in place during winter pruning. However higher bud numbers did result in consistently smaller bunches across three seasons.

Experimental results also suggested that small bunches may develop more intense colour independently of fruit load and that colour development was not inhibited in heavier cropping vines. Although this is only a single marker of quality, it suggests that the current industry view that heavy crops result in poorer quality wine is a reflection of seasonal effects on bunch size. That is, in years with a light crop load,

bunch size will be smaller and quality correspondingly higher. Thus, bunch thinning or heavy pruning to obtain a light crop may have either no effect on quality or an effect opposite to that expected, if either activity results in higher average bunch weight. This result may explain the confusion in the literature, with conflicting claims about crop load influences on wine quality. A re-evaluation of published material or experimental approach, is suggested where possible using bunch size rather than total yield as the controlling variable.

Application of these results in commercial vineyards may require further work to establish a detailed management approach, but broad guidelines can be drawn as follows:

- Bunch size is of major importance in managing for quality and yield targets.
- Dormant pruning to a high bud number will reduce average bunch size, relative to lighter cropped vines, but the resultant bunch size will be highly dependent on spring weather.
- Target yields set for wine quality need to take bunch size into account and bunch thinning should target larger bunches for removal.
- Higher yields may not preclude high quality wine, provided the higher yield is as a result of increased bunch number (pruning) and not bunch size (weather).

Further research is required to clarify several of the issues raised here. With yield targets driven by assumptions about yield and quality, it is critically important to confirm the bunch size / colour relationship and establish whether it extends to other

quality parameters. Similarly the fungicide toxicity issue needs to be further clarified. While any negative effects on fruitset may be seen as contributing to yield variability, a small reduction in bunch size might be seen as desirable.

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Appendix 1:

Weather data for sites 1-6 in Chapter 2.

Sites 1 and 6			
Year	Mean maximum temperature for November	Mean sunshine hours for November	Rainfall intensity for November
1988	18.9	6.9	2.8
1989	19.4	8.1	6.8
1990	20.1	8.5	2.4
1991	18.7	7.1	2.6
1992	18.1	6.4	1.7
1993	18.4	7.7	4.7
1994	19	6.6	5.1
1995	18	7.2	7.8
1996	17.9	7.7	6.5
1997	20	9.0	2
1998	18.2	7.1	1.5
1999	18.5	9.0	2

Sites 3 and 5			
Year	Mean maximum temperature for November	Mean sunshine hours for November	Rainfall intensity for November
1988	19.1	6.4	
1989	20.5	6.8	5.1
1990	19.2	9.8	12.6
1991	18.6	9.2	5.4
1992	17.9	7.9	3.4
1993			4.1
1994	18.8	7.9	5.9
1995	17.8	5.8	11.7
1996	16.8	6.9	1.5
1997	19.9	6.9	14.2
1998	19.0	6.9	4.1
1999	18.4	7.0	4.4

Site 4			
Year	Mean maximum temperature for November	Mean sunshine hours for November	Rainfall intensity for November
1988	18.8	8.5	
1989	19.7	8.1	2.6
1990	20.5	8.1	7.6
1991	19.2	8.1	4.1
1992	18	6.1	7.1
1993	19.1		1.7
1994	19.6	8.2	11.9
1995	18	7.7	7.4
1996	18.3	8.7	4.1
1997	21.3	8.7	6.9
1998	18.6	8.7	2.2
1999	18.5	8.4	26.6

Site 2			
Year	Mean maximum temperature for November	Mean sunshine hours for November	Rainfall intensity for November
1988	19.9	6.3	3.2
1989	26.2	5.1	4.1
1990	20.1	5.7	3.3
1991	18.1	7.2	3.9
1992	18.6	5.9	2.4
1993	18.1	7.1	2.9
1994	18.2		3.7
1995	17.6		8.6
1996	17.5		14.4
1997	19.8	7.5	1.5
1998	18.1	6.9	1.5
1999	18.1	8.1	4.2

Weather data for sites 2 and 6 for the vintages 2003, 2002 and 2001.

Vintage	Site 2		Site 6	
	Rainfall at flowering	Sunshine Hours at flowering	Mean maximum temperature at flowering	Sunshine hours at flowering
2003	13	7.7	20.7	9
2002	50	4.9	17.3	6.8
2001	58	9.1	20.1	9.9

Appendix 2:

Leaf Nutrient analysis: The total acid extractable phosphorous, boron and molybdenum of leaf lamina was determined by using ICP methods.

Nutrient levels (ppm)		
	Site 2	Site 6
Phosphorous	3850	5340
Boron	29.7	32.6
Molybdenum	0.13	0.14

Appendix 3:

Pollen viability when tested in vitro in different sucrose concentrations.

Sucrose concentration (%)	Pollen Viability (%)
5	38.8
10	47.1
20	38.7
30	31.3

Appendix 4:

Yield component measurements in 2003 and 2004 in response to the fruit load treatment (Trial 2, Chapter 4).

	2003			2004		
	Berry number	Berry weight	Bunch weight	Berry number	Berry weight	Bunch weight
6 tonnes/ha	105	0.99	103.05	172	0.90	154.66
10 tonnes/ha	107	0.97	104.41	170	0.93	158.43
14 tonnes/ha	94	1.00	93.46	156	0.97	150.37
P=0.05	ns	ns	ns	ns	ns	ns

Yield component measurements in 2003 and 2004 in response to the canopy pruning treatment (Trial 1, Chapter 4).

	2003			2004		
	Berry number	Berry weight	Bunch weight	Berry number	Berry weight	Bunch weight
20 buds	101	1.01	101.63	166	0.94	155.57
30 buds	103	0.99	103.13	172	0.92	158.69
40 buds	100	0.96	96.16	160	0.94	149.21
P=0.05	ns	ns	ns	ns	ns	ns